EXHIBIT A

US005246925A

United States Patent [19]

DeLuca et al.

[11] Patent Number: 5,246,925

[45] Date of Patent: Sep. 21, 1993

[54] **19-NOR-VITAMIN D COMPOUNDS FOR USE IN TREATING HYPERPARATHYROIDISM**

[75] Inventors: **Hector F. DeLuca**, Deerfield; **Heinrich K. Schnoes; Kato L. Perlman**, both of Madison, all of Wis.; **Rafal R. Sicinski**, Warsaw, Poland; **Jean M. Prahl**, Madison, Wis.

[73] Assignee: **Wisconsin Alumni Research Foundation**, Madison, Wis.

[21] Appl. No.: **960,241**

[22] Filed: **Oct. 13, 1992**

Related U.S. Application Data

[60] Continuation of Ser. No. 879,706, May 5, 1992, abandoned, which is a continuation of Ser. No. 557,400, Jul. 23, 1990, abandoned, which is a division of Ser. No. 481,354, Feb. 16, 1990, which is a continuation-in-part of Ser. No. 321,030, Mar. 9, 1989, abandoned.

[51] **Int. Cl.**[5] **A61K 31/59**; A61K 31/695

[52] **U.S. Cl.** **514/167**; 514/63

[58] **Field of Search** 514/167, 63; 552/653

[56] **References Cited**

U.S. PATENT DOCUMENTS

4,769,181 9/1988 DeLuca et al. 552/653

OTHER PUBLICATIONS

Chemical Abstracts (110:19004r) 1989.

Primary Examiner—Frederick E. Waddell
Assistant Examiner—K. Weddington
Attorney, Agent, or Firm—Andrus, Sceales, Starke & Sawall

[57] **ABSTRACT**

This invention provides a novel class of vitamin D-related compounds, namely the 1α-hydroxy-19-nor-vitamin D analogs, as well as a general method for their chemical synthesis. The compounds exhibit pronounced activity in arresting the proliferation of undifferentiated cells, including malignant cells, and in inducing their differentiation, and thus represent novel therapeutic agents for the treatment of malignant and other diseases characterized by the proliferative growth of undifferentiated cells. Formulations for therapeutic use and treatment methods are also provided.

9 Claims, No Drawings

19-NOR-VITAMIN D COMPOUNDS FOR USE IN TREATING HYPERPARATHYROIDISM

This invention was made with United States government support awarded by the Department of Health and Human Services (NIH), Grant number: DK-14881. The United States Government has certain rights in this invention.

This application is a continuation of application Ser. No. 07/879,706 filed May 5, 1992, now abandoned, which is a continuation of application Ser. No. 07/557,400 filed Jul. 23, 1990, now abandoned, which is a division of application Ser. No. 07/481,354 pending filed Feb. 16, 1990, which is a continuation-in-part of application Ser. No. 07/321,030 filed Mar. 9, 1989, now abandoned.

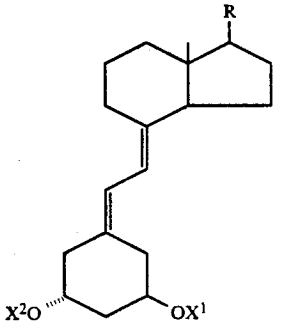
This invention relates to biologically active vitamin D compounds. More specifically, the invention relates to 19-nor-analogs of 1α-hydroxylated vitamin D compounds and to a general process for their preparation.

BACKGROUND

The 1α-hydroxylated metabolites of vitamin D—most importantly 1α,25-dihydroxyvitamin D_3 and 1α,25-dihydroxyvitamin D_2—are known as highly potent regulators of calcium homeostasis in animals and humans, and more recently their activity in cellular differentiation has also been established. As a consequence, many structural analogs of these metabolites, such as compounds with different side chain structures, different hydroxylation patterns, or different stereochemistry, have been prepared and tested. Important examples of such analogs are 1α-hydroxyvitamin D_3, 1α-hydroxyvitamin D_2, various side chain fluorinated derivatives of 1α,25-dihydroxyvitamin D_3, and side chain homologated analogs. Several of these known compounds exhibit highly potent activity in vito or in vitro, and possess advantageous activity profiles and thus are in use, or have been proposed for use, in the treatment of a variety of diseases such as renal osteodystrophy, vitamin D-resistant rickets, osteoporosis, psoriasis, and certain malignancies.

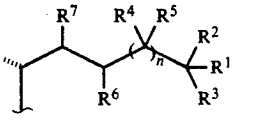
DISCLOSURE AND DESCRIPTION OF THE INVENTION

A class of 1α-hydroxylated vitamin D compounds not known heretofore are the 19-nor-analogs, i.e. compounds in which the ring A exocyclic methylene group (carbon 19) typical of all vitamin D system has been removed and replaced by two hydrogen atoms. Structurally these novel analogs are characterized by the general formula I shown below:



I

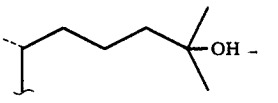
where X^1 and X^2 are each selected from the group consisting of hydrogen and acyl, and where the group R represents any of the typical side chains known for vitamin D type compounds. Thus, R may be an alkyl, hydrogen, hydroxyalkyl or fluoroalkyl group, or R may represent the following side chain:



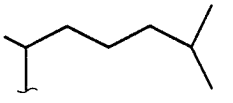
wherein R^1 represents hydrogen, hydroxy or O-acyl, R^2 and R^3 are each selected from the group consisting of alkyl, hydroxyalkyl and fluoroalkyl, or, when taken together represent the group $—(CH_2)_m—$ where m is an integer having a value of from 2 to 5, R^4 is selected from the group consisting of hydrogen, hydroxy, fluorine, O-acyl, alkyl, hydroxyalkyl and fluoroalkyl, R^5 is selected from the group consisting of hydrogen, fluorine, alkyl, hydroxyalkyl and fluoroalkyl, or, R^4 and R^5 taken together represent double-bonded oxygen, R^6 and R^7 are each selected from the group consisting of hydrogen, hydroxy, O-acyl, fluorine and alkyl, or, R^6 and R^7 taken together form a carbon-carbon double bond, and wherein n is an integer having a value of from 1 to 5, and wherein the carbon at any one of positions 20, 22, or 23 in the side chain may be replaced by an O, S, or N atom.

Specific important examples of side chains are the structures represented by formulas (a), (b), (c), (d) and (e) below, i.e. the side chain as it occurs in 25-hydroxyvitamin D_3 (a); vitamin D_3 (b); 25-hydroxyvitamin D_2 (c); vitamin D_2 (d); and the C-24-epimer of 25-hydroxyvitamin D_2 (e).

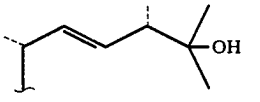
(a)



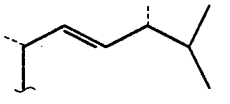
(b)

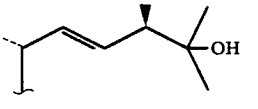


(c)



(d)

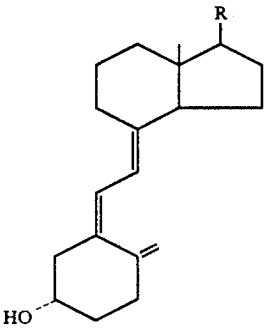




In this specification and the claims, the term 'alkyl' signifies an alkyl radical of 1 to 5 carbons in all isomeric forms, such as methyl, ethyl, propyl, isopropyl, butyl, isobutyl, pentyl, etc., and the terms 'hydroxyalkyl' and 'fluoroalkyl' refer to such an alkyl radical substituted by one or more hydroxy or fluoro groups respectively, and

the term 'acyl' means an aliphatic acyl group of 1 to 5 carbons, such as formyl, acetyl, propionyl, etc. or an aromatic acyl group such as benzoyl, nitrobenzoyl or halobenzoyl. The term 'aryl' signifies a phenyl-, or an alkyl-, nitro- or halo-substituted phenyl group.

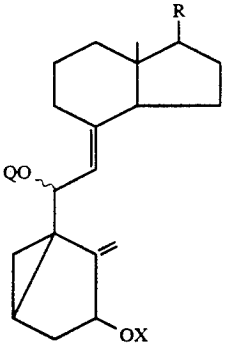
The preparation of b 1α-hydroxy-19-nor-vitamin D compounds having the basic structure shown above can be accomplished by a common general method, using known vitamin D compounds as starting materials. Suitable starting materials are, for example, the vitamin D compounds of the general structure II:



II

where R is any of the side chains as defined above. These vitamin D starting materials are known compounds, or compounds that can be prepared by known methods.

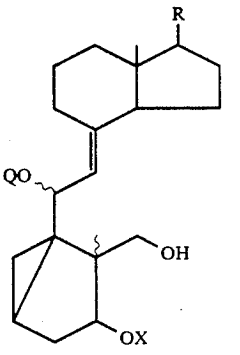
Using the procedure of DeLuca et al. (U.S. Pat. No. 4,195,027), the starting material is converted to the corresponding 1α-hydroxy-3,5-cyclovitamin D derivative, having the general structure III below, where X represents hydrogen and Q represents an alkyl, preferably methyl:



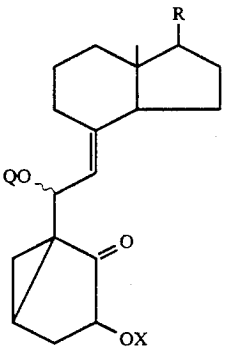
III

So as to preclude undesired reaction of the 1α-hydroxy group in subsequent steps, the hydroxy group is converted to the corresponding acyl derivative, i.e. the compound III shown above, where X represents an acyl group, using standard acylation procedures, such as treatment with an acyl anhydride or acyl halide in pyridine at room temperature or slightly elevated temperature (30°-70° C.). It should be understood also that whereas the process of this invention is illustrated here with acyl protection of hydroxy functions, alternative standard hydroxy-protecting groups can also be used, such as, for example, alkylsilyl or alkoxyalkyl groups. Such protecting groups are well-known in the art (e.g. trimethylsilyl, triethylsilyl, t.-butyldimethylsilyl, or tetrahydrofuranyl, methoxymethyl), and their use is considered a routine modification of experimental detail within the scope of the process of this invention.

The derivative as obtained above is then reacted with osmium tetroxide, to produce the 10,19-dihydroxy analog, IV (where X is acyl), which is subjected to diol cleavage using sodium metaperiodate or similar vicinal diol cleavage reagents (e.g. lead tetraacetate) to obtain the 10-oxo-intermediate, having the structure V below (where X is acyl):



IV

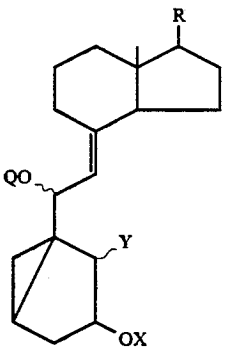


V

These two consecutive steps can be carried out according to the procedures given by Paaren et al. [J. Org. Chem. 48, 3819 (1983)]. If the side chain unit, R, carries vicinal diols (e.g. 24,25-dihydroxy- or 25,26-dihydroxy, etc.), these, of course, also need to be protected, e.g. via acylation, silylation, or as the isopropylidene derivative prior to the periodate cleavage reactions.

In most cases, the acylation of the 1α-hydroxy group as mentioned above will simultaneously effect the acylation of side chain hydroxy functions, and these acylation conditions can, of course, be appropriately adjusted (e.g. elevated temperatures, longer reaction times) so as to assure complete protection of side chain vicinal diol groupings.

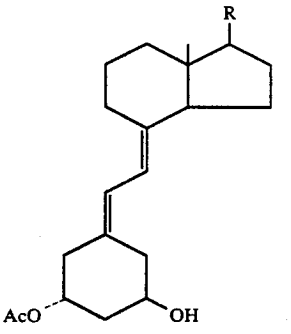
The next step of the process comprises the reduction of the 10-oxo-group to the corresponding 10-alcohol having the structure VI shown below (where X is acyl and Y represents hydroxy). When X is acyl, this reduction is carried out conveniently in an organic solvent at from about 0° C. to about room temperature, using $NaBH_4$ or equivalent hydride reducing agents, selective for the reduction of carbonyl groups without cleaving ester functions. Obviously, when X is a hydroxy-protecting group that is stable to reducing agents, any of the other hydride reducing agents (e.g. $LiAlH_4$, or analogous reagents) may be employed also.



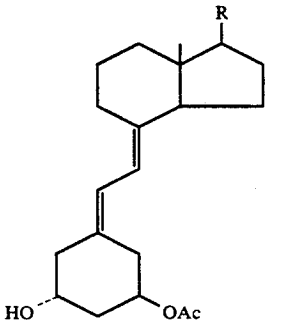
VI

The 10-hydroxy intermediate is then treated with an alkyl- or arylsulfonylhalide (e.g. mathanesulfonylchloride) in a suitable solvent (e.g. pyridine) to obtain the corresponding 10-O-alkyl-or arylsulfonyl derivative (the compound having the structure shown VI above, where Y is alkyl—SO_2O—, or aryl—SO_2O—, and this sulfonate intermediate is then directly reduced, with lithium aluminum hydride, or the analogous known lithium aluminum alkyl hydride reagents in an ether solvent, at a temperature ranging from 0° C. to the boiling temperature of the solvent, thereby displacing the sulfonate group and obtaining the 10-deoxy derivative, represented by the structure VI above, where X and Y are both hydrogen. As shown by the above structure, a 1-O-acyl function in the precursor compound V is also cleaved in this reduction step to produce the free 1α-hydroxy function, and any O-acyl protecting group in the side chain would, of course, likewise be reduced to the corresponding free alcohol function, as is well understood in the art. If desired, the hydroxy groups at C-1 (or hydroxy groups in the side chain) can be re-protected by acylation or silylation or ether formation to the corresponding acyl, alkylsilyl or alkoxyalkyl derivative, but such protection is not required. Alternative hydroxy-protecting groups, such as alkylsilyl or alkoxyalkyl groups would be retained in this reduction step, but can be removed, as desired, at this or later stages in the process by standard methods known in the art.

The above 1α-hydroxy-10-deoxy cyclovitamin D intermediate is next solvolyzed in the presence of a low-molecular weight organic acid, using the conditions of DeLuca et al. (U.S. Pat. Nos. 4,195,027 and 4,260,549). When the solvolysis is carried out in acetic acid, for example, there is obtained a mixture of 1α-hydroxy-19-nor-vitamin D 3-acetate and 1α-hydroxy-19-nor-vitamin D 1-acetate (compounds VII and VIII, below), and the analogous 1- and 3-acylates are produced, when alternative acids are used for solvolysis.



VII



VIII

Direct basic hydrolysis of this mixture under standard conditions then produces the desired 1α-hydroxy-19-nor-vitamin D compounds of structure I above (where X^1 and X^2 are hydrogen). Alternatively, the above mixture of monacetates may also be separated (e.g. by high pressure liquid chromatography) and the resulting 1-acetate and 3-acetate isomers may be subjected separately to hydrolysis to obtain the same final product from each, namely the 1α-hydroxy-19-nor-vitamin D compounds of structure I. Also the separated monoacetates of structure VII or VIII or the free 1,3-dihydroxy compound can, of course, be reacylated according to standard procedures with any desired acyl group, so as to produce the product of structure I above, where X^1 and X^2 represent acyl groups which may be the same or different.

Biological Activity of 1α-Hydroxy-19-Nor-Vitamin D Compounds

The novel compounds of this invention exhibit an unexpected pattern of biological activity, namely high potency in promoting the differentiation of malignant cells and little or no activity in calcifying bone tissue. This is illustrated by the biological assay results obtained for 1α,25-dihydroxy-19-nor-vitamin D_3 (compounds Ia), which are summarized in Tables 1 and 2, respectively. Table 1 shows a comparison of the activity of the known active metabolite 1α,25-dihydroxyvitamin D_3 and the 19-nor analog (Ia) in inducing the differentiation of human leukemia cells (HL-60 cells) in culture to normal cells (monocytes). Differentiation activity was assessed by three standard differentiation assays, abbreviated in Table 1 as NBT (nitroblue tetrazolium reduction), NSE (non-specific esterase activity), and PHAGO (phagocytosis activity). The assays were conducted according to known procedures, as given, for example, by DeLuca et al. (U.S. Pat. No. 4,717,721) and Ostrem et al., J. Biol. Chem. 262, 14164, 1987). For each assay, the differentiation activity of the test compounds is expressed in terms of the percent of HL-60 cells hav-

ing differentiated to normal cells in response to a given concentration of test compound.

The results summarized in Table 1 clearly show that the new analog, 1α,25-dihydroxy-19-nor-vitamin D_3 (Ia) is as potent as 1α,25-dihydroxyvitamin D_3 in promoting the differentiation of leukemia cells. Thus in all three assays close to 90% of the cells are induced to differentiate by 1α,25-dihydroxy-vitamin D_3 at a concentration of 1×10^{-7} molar, and the same degree of differentiation (i.e. 90, 84 and 90%) is achieved by the 19-nor analog (Ia).

TABLE 1

	Differentiation of HL-60 Cells		
	% Differentiated Cells (mean ± SEM)		
	NBT	NSE	PHAGO
1α,25-dihydroxyvitamin D_3 (moles/liter)			
1×10^{-7}	86 ± 2	89 ± 1	87 ± 3
1×10^{-8}	60 ± 2	60 ± 3	64 ± 2
1×10^{-9}	33 ± 2	31 ± 2	34 ± 1
1α,25-Dihydroxy-19-nor-vitamin D_3, (Ia) (moles/liter)			
2×10^{-7}	94 ± 2	95 ± 3	94 ± 2
1×10^{-7}	90 ± 4	84 ± 4	90 ± 4
5×10^{-8}	72 ± 3	73 ± 3	74 ± 3
1×10^{-8}	61 ± 3	60 ± 3	56 ± 1
1×10^{-9}	32 ± 1	31 ± 1	33 ± 1

In contrast to the preceding results, the new 19-nor analog (Ia) exhibits no activity in an assay measuring the calcification of bone, a typical response elicited by vitamin D compounds. Relevant data, representing the results of an assay comparing the bone calcification activity in rats of 1α,25-dihydroxyvitamin D_3 and 1α,25-dihydroxy-19-nor-vitamin D_3 (Ia), are summarized in Table 2. This assay was conducted according to the procedure described by Tanaka et al., Endocrinology 92, 417 (1973).

The results presented in Table 2 show the expected bone calcification activity of 1α,25-dihydroxyvitamin D_3 as reflected by the increase in percent bone ash, and in total ash at all dose levels. In contrast, the 19-nor analog Ia exhibits no activity at all three dose levels, when compared to the vitamin D-deficient (-D) control group.

TABLE 2

	Calcification Activity		
Compound	Amount Administered* (pmoles/day/7 days)	% Ash (mean ± SEM)	Total Ash (mg) (mean ± SEM)
-D (control)	0	19 ± 0.8	23 ± 1.2
1α,25-dihydroxy-vitamin D_3	32.5	23 ± 0.5	34 ± 1.6
	65.0	26 ± 0.7	36 ± 1.1
	325.0	28 ± 0.9	40 ± 1.9
1α,25-dihydroxy-19-nor-vitamin D_3 (Ia)	32.5	22 ± 0.9	28 ± 1.6
	65.0	19 ± 1.5	28 ± 3.4
	325.0	19 ± 1.2	30 ± 2.4

*Each assay group comprised 6 rats, receiving the indicated amount of test compound by intraperitoneal injection daily for a period of seven days.

Thus the new 19-nor analog shows a selective activity profile combining high potency in inducing the differentiation of malignant cells with very low or no bone calcification activity. The compounds of this novel structural class, therefore, can be useful as therapeutic agents for the treatment of malignancies. Because the differentiative activity of vitamin D compounds on keratinocytes of skin (Smith et al., J. Invest. Dermatol. 86, 709, 1986; Smith et al., J. Am. Acad. Dermatol. 19, 516, 1988) is believed to be an indication of successful treatment of psoriasis (Takamoto et al., Calc. Tissue Int. 39, 360, 1986), these compounds should prove useful in treating this and other skin disorders characterized by proliferation of undifferentiated skin cells. These compounds should also find use in the suppression of parathyroid tissue, as for example, in cases of secondary hyperparathyroidism found in renal disease (Slatopolsky et al., J. Clin. Invest. 74, 2136, 1984).

For treatment purposes, the novel compounds of this invention can be formulated as solutions in innocuous solvents, or as emulsions, suspensions or dispersions in suitable innocuous solvents or carriers, or as pills, tablets or capsules, containing solid carriers according to conventional methods known in the art. For topical applications the compounds are advantageously formulated as creams or ointments or similar vehicle suitable for topical applications. Any such formulations may also contain other pharmaceutically-acceptable and non-toxic excipients such as stabilizers, anti-oxidants, binders, coloring agents or emulsifying or taste-modifying agents.

The compounds are advantageously administered by injection, or by intravenous infusion of suitable sterile solutions, or in the form of oral doses via the alimentary canal, or topically in the form of ointments, lotions, or in suitable transdermal patches. For the treatment of malignant diseases, the 19-nor-vitamin D compounds of this invention are administered to subjects in dosages sufficient to inhibit the proliferation of malignant cells and induce their differentiation into normal monocyte-macrophages. Similarly, for the treatment of psoriasis, the compounds may be administered orally or topically in amounts sufficient to arrest the proliferation of undifferentiated keratinocytes, and in the treatment of hyperparathyroidism, the compounds are administered in dosages sufficient to suppress parathyroid activity, so as to achieve parathyroid hormone levels in the normal range. Suitable dosage amounts are from 1 to 500 μg of compound per day, such dosages being adjusted, depending on diseases to be treated, its severity and the response or condition of the subject as well-understood in the art.

This invention is more specifically described by the following illustrative examples. In these examples specific products identified by Roman numerals and letters, i.e. Ia, Ib, . . . , IIa, IIb, . . . , etc. refer to the specific structures and side chain combinations identified in the preceding description.

EXAMPLE 1

Preparation of 1α,25-dihydroxy-19-nor-vitamin D_3 (Ia)

(a) 1α,25-Dihydroxy-3,5-cyclovitamin D_3 1-acetate, 6-methyl ether:

Using 25-hydroxyvitamin D_3 (IIa) as starting material, the known 1α,25-dihydroxy-3,5-cyclovitamin D_3 derivative IIIa (X=H) was prepared according to published procedures (DeLuca et al., U.S. Pat. No. 4,195,027 and Paaren et al., J. Org. Chem. 45, 3252 (1980)). This product was then acetylated under standard conditions to obtain the corresponding 1-acetate derivative IIIa (X=Ac).

(b) 10,19-Dihydro-1α,10,19,25-tetrahydroxy-3,5-cyclovitamin D_3 1-acetate, 6-methyl ether (IVa):

Intermediate IIIa (X=Ac) was treated with a slight molar excess of osmium tetroxide in pyridine according to the general procedure described by Paaren et al. (J. Org. Chem. 48, 3819 (1983)) to obtain the 10,19-dihydroxylated derivative IVa. Mass spectrum m/z (relative intensity), 506 (M^+, 1), 488 (2), 474 (40), 425 (45), 396 (15), 285 (5), 229 (30), 133 (45), 59 (80), 43 (100). ^1H NMR ($CDCl_3$) δ 0.52 (3H, s, 18—CH_3), 0.58 (1H, m, 3—H), 0.93 (3H, d, J=6.1 Hz, 21—CH_3), 1.22 (6H, s, 26—CH_3 and 27—CH_3), 2.10 (3H, s, $COCH_3$), 3.25 (3H, s, 6—OCH_3), 3.63 (2H, m, 19—CH_2), 4.60 (1H, d, J=9.2 Hz, 6—H), 4.63 (1H, dd, 1β—H), 4.78 (1H, d, J=9.2 Hz, 7—H).

(c) 1α,25-Dihydroxy-10-oxo-3,5-cyclo-19-nor-vitamin D_3 1-acetate, 6-methyl ether (Va):

The 10,19-dihydroxylated intermediate IVa was treated with a solution of sodium metaperiodate according to the procedure given by Paaren et al. (J. Org. Chem. 48, 3819, 1983) to produce the 10-oxo-cyclovitamin D derivative (Va, X=Ac). Mass spectrum m/z (relative intensity) 442 (M^+-MeOH) (18), 424 (8), 382 (15), 364 (35), 253 (55), 225 (25), 197 (53), 155 (85), 137 (100). ^1H NMR ($CDCl_3$) δ 0.58 (3H, s, 18—CH_3), 0.93 (3H, d, J=6.6 Hz, 21—CH_3), 1.22 (6H, s, 26—CH_3 and 27—CH_3), 2.15 (s, 3—$OCOCH_3$), 3.30 (3H, s, 6-OCH_3), 4.61 (1H, d, J=9.1 Hz, 6-H), 4.71 (1H, d, J=9.6 Hz, 7-H), 5.18 (1H, m, 1β-H).

It has been found also that this diol cleavage reaction does not require elevated temperatures, and it is, indeed, generally preferable to conduct the reaction at approximately room temperature.

(d) 1α-Acetoxy-10,25-dihydroxy-3,5-cyclo-19-nor-vitamin D_3 6-methyl ether (VIa, X=Ac, Y=OH):

The 10-oxo derivative Va (X=Ac) (2.2 mg, 4.6 μmol) was dissolved in 0.5 ml of ethanol and to this solution 50 μl (5.3 μmol) of a $NaBH_4$, solution (prepared from 20 mg of $NaBH_4$, 4.5 ml water and 0.5 ml of 0.01 N NaOH solution) was added and the mixture stirred at 0° C. for ca. 1.5 h, and then kept at 0° C. for 16 h. To the mixture ether was added and the organic phase washed with brine, dried over $MgSO_4$, filtered and evaporated. The crude product was purified by column chromatography on a 15×1 cm silica gel column and the alcohol VIa (X=Ac, Y=OH) was eluted with ethyl acetate hexane mixtures to give 1.4 mg (3 μmol) of product. Mass spectrum m/z (relative intensity) 476 (M^+) (1), 444 (85), 426 (18), 384 (30), 366 (48), 351 (21), 255 (35), 237 (48), 199 (100), 139 (51), 59 (58).

(e) 1α,25-Dihydroxy-19-nor-vitamin D_3 (Ia, X^1=X^2=H):

The 10-alcohol (VIa, X=Ac, Y=OH) (1.4 mg) was dissolved in 100 μl anhydrous CH_2Cl_2 and 10 μl (14 μmol) triethylamine solution [prepared from 12 mg (16 μl) triethylamine in 100 μl anhydrous CH_2Cl_2], followed by 7 μl (5.6 μmol) mesyl chloride solution (9 mg mesyl chloride, 6.1 μl, in 100 μl anhydrous CH_2Cl_2) added at 0° C. The mixture was stirred at 0° C. for 2 h. The solvents were removed with a stream of argon and the residue (comprising compound VIa, X=Ac, Y=CH_3SO_2O-)dissolved in 0.5 ml of anhydrous tetrahydrofuran; 5 mg of $LiAlH_4$ was added at 0° C. and the mixture kept at 0° C. for 16 h. Excess $LiAlH_4$ was decomposed with wet ether, the ether phase was washed with water and dried over $MgSO_4$, filtered and evaporated to give the 19-nor product VIa (X=Y=H).

This product was dissolved in 0.5 ml of acetic acid and stirred at 55° C. for 20 min. The mixture was cooled, ice water added and extracted with ether. The other phase was washed with cold 10% sodium bicarbonate solution, brine, dried over $MgSO_4$, filtered and evaporated to give the expected mixture of 3-acetoxy-1α-hydroxy- and 1α-acetoxy-3-hydroxy isomers, which were separated and purified by HPLC (Zorbax Sil column, 6.4×25 cm, 2-propanol in hexane) to give about 70 μg each of compounds VIIa and XIIIa. UV (in EtOH) λ_{max} 242.5 (OD 0.72), 251.5 (OD 0.86), 260 (OD 0.57).

Both 19-nor-1,25-dihydroxyvitamin D_3 acetates VIIa and VIIIa were hydrolyzed in the same manner. Each of the monoacetates was dissolved in 0.5 ml of ether and 0.5 ml 0.1N KOH in methanol was added. The mixture was stirred under argon atmosphere for 2 h. More ether was added and the organic phase washed with brine, dried over anhydrous $MgSO_4$, filtered and evaporated. The residue was dissolved in a 1:1 mixture of 2-propanol and hexane and passed through a Sep Pak column and washed with the same solvent. The solvents were evaporated and the residue purified by HPLC (Zorbax Sil, 6.4×25 cm, 10% 2-propanol in hexane). The hydrolysis products of VIIa and VIIIa were identical and gave 66 μg of Ia (X^1=X^2=H). Mass spectrum (m/z relative intensity) 404 (M^+) (100), 386 (41), 371 (20), 275 (53), 245 (51), 180 (43), 135 (72), 133 (72), 95 (82), 59 (18), exact mass calcd. for $C_{26}H_{44}O_3$ 404.3290, found 404.3272. ^1H NMR ($CDCl_3$) δ 0.52 (3H, s, 18—CH_3), 0.92 (3H, d, J=6.9 Hz, 21—CH_3), 1.21 (6H, s, 26—CH_3 and 27—CH_3), 4.02 (1H, m, 3α-H), 4.06 (1H, m, 1β-H), 5.83 (1H, d, J=11.6 Hz, 7-H), 6.29 (1H, d, J=10.7 Hz, 6-H). UV (in EtOH), λ_{max} 243 (OD 0.725), 251.5 (OD 0.823), 261 (OD 0.598).

EXAMPLE 2

Preparation of 1α-hydroxy-19-nor-vitamin D_3 (Ib)

(a) With vitamin D_3 (IIb) as starting material, and utilizing the conditions of Example 1a, there is obtained known 1α-hydroxy-3,5-cyclovitamin D_3 1-acetate, 6-methyl ether, compound IIIb (X=Ac).

(b) By subjecting intermediate IIIb (X=Ac), as obtained in Example 2a above to the conditions of Example 1b, there is obtained 10,19-dihydro-1α,10,19-trihydroxy-3,5-cyclovitamin D_3 1-acetate, 6-methyl ether IVb (X=Ac).

(c) By treatment of intermediate IVb (X=Ac) with sodium metaperiodate according to Example 1c above, there is obtained 1α-hydroxy-10-oxo-3,5-cyclo-19-nor-vitamin D_3 1-acetate, 6-methyl ether Vb (X=Ac).

(d) Upon reduction of the 10-oxo-intermediate Vb (X=Ac) under the conditions of Example 1d above, there is obtained 1α-acetoxy-10-hydroxy-3,5-cyclo-19-nor-vitamin D_3 6-methyl ether VIb (X=Ac, Y=OH).

(e) Upon processing intermediate VIb (X=Ac, Y=OH) through the procedure given in Example 1e above, there is obtained 1α-hydroxy-19-nor-vitamin D_3 (Ib, X^1=X^2=H).

EXAMPLE 3

Preparation of 1α,25-dihydroxy-19-nor-vitamin D_2

(a) Utilizing 25-hydroxyvitamin D_2 (IIc) as starting material and experimental conditions analogous to those of Example 1a, there is obtained 1α,25-dihydroxy-3,5-cyclovitamin D_2 1-acetate, 6-methyl ether, compound IIIc (X=Ac).

(b) Subjecting intermediate IIId (X=Ac), as obtained in Example 3a above, to the reaction conditions of Example Ib, provides 10,19-dihydro-1α,10,19,25-tetrahydroxy-3,5-cyclovitamin vitamin D_2 1-acetate, 6-methyl ether, IVc (X=Ac).

(c) By treatment of intermediate IVc (X=Ac) with sodium metaperiodate according to general procedures of Example 1c above, there is obtained 1α,25-dihydroxy-10-oxo-3,5-cyclo-19-nor-vitamin D_2 1-acetate, 6-methyl ether Vc (X=Ac).

(d) Upon reduction of the 10-oxo-intermediate Vc (X=Ac) under conditions analogous to those of Example 1d above, there is obtained 1α-acetoxy-10,25-dihydroxy-3,5-cyclo-19-nor-vitamin D_2 6-methyl ether VIc (X=Ac, Y=OH).

(e) Upon processing intermediate VIc (X=Ac, Y=OH) through the procedural steps given in Example 1e above, there is obtained 1α,25-dihydroxy-19-nor-vitamin D_2 (Ic, X^1=X^2=H).

EXAMPLE 4

Preparation of 1α-hydroxy-19-nor-vitamin D_2

(a) With vitamin D_2 (IId) as starting material, and utilizing the conditions of Example 1a, there is obtained known 1α-hydroxy-3,5-cyclovitamin D_2 1-acetate, 6-methyl ether, compound IIId (X=Ac).

(b) By subjecting intermediate IIId (X=Ac), as obtained in Example 4a above to the conditions of Example 1b, there is obtained 10,19-dihydro-1α,10,19-trihydroxy-3,5-cyclovitamin D_2 1-acetate, 6-methyl ether, IVd (X=Ac).

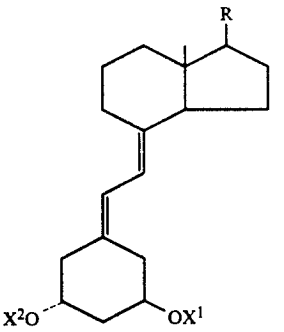
(c) By treatment of intermediate IVb (X=Ac) with sodium metaperiodate according to Example 1c above, there is obtained 1α-hydroxy-10-oxo-3,5-cyclo-19-nor-vitamin D_2 1-acetate, 6-methyl ether, Vd (X=Ac).

(d) Upon reduction of the 10-oxo-intermediate Vd (X=Ac) under the conditions of Example 1d above, there is obtained 1α-acetoxy-10-hydroxy-3,5-cyclo-19-nor-vitamin D_2 6-methyl ether, VId (X=Ac, Y=OH).

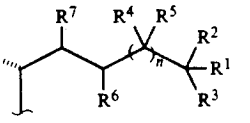
(e) Upon processing intermediate VId (X=Ac, Y=OH) through the procedure given in Example 1e above, there is obtained 1α-hydroxy-19-nor-vitamin D_2 (Id, X^1=X^2=H).

We Claim:

1. A method for treating hyperparathyroidism which comprises suppressing parathyroid activity by administering to a patient having such a disorder at least one compound having the formula



where X^1 and X^2 are each selected from the group consisting of hydrogen, acyl, alkylsilyl and alkoxyalkyl, and where R is selected from the group consisting of alkyl, hydrogen, hydroxyalkyl, fluoroalkyl and a side chain of the formula



where R^1 represents hydrogen, hydroxy or O-acyl, R^2 and R^3 are each selected from the group consisting of alkyl, hydroxyalkyl and fluoroalkyl, or, when taken together represent the group $-(CH_2)_m-$ where m is an integer having a value of from 2 to 5, R^4 is selected from the group consisting of hydrogen, hydroxy, fluorine, O-acyl, alkyl, hydroxyalkyl and fluoroalkyl, R^5 is selected from the group consisting of hydrogen, fluorine, alkyl, hydroxyalkyl and fluoroalkyl, or, R^4 and R^5 taken together represent double-bonded oxygen, R^6 and R^7 are each selected from the group consisting of hydrogen, hydroxy, O-acyl, fluorine and alkyl, or, R^6 and R^7 taken together form a carbon-carbon double bond, and wherein n is an integer having a value of from 1 to 5 and wherein the carbon at any one of positions 20, 22, or 23 in the side chain may be replaced by an O, S, or N atom, with the proviso that when n is 1 then R^2 and R^3 must both be methyl, said at least one compound administered by oral, topical or parenteral means in an amount from about 1 μg to about 500 μg per day to the patient sufficient to suppress parathyroid activity thereby treating renal osteodystrophy.

2. The method of claim 1 wherein said at least one compound is administered together with a pharmaceutically acceptable excipient.

3. The method of claim 1 wherein said at least one compound is in a solid or liquid vehicle ingestible by and non-toxic to the patient.

4. The method of claim 1 where the said at least one compound is 1α,25-dihydroxy-19-nor-vitamin D_3.

5. The method of claim 1 where the said at least one compound is 1α-hydroxy-19-nor-vitamin D_3.

6. The method of claim 1 where the said at least one compound is 1α,25-dihydroxy-19-nor-vitamin D_2.

7. The method of claim 1 where the said at least one compound is 1α-hydroxy-19-nor-vitamin D_2.

8. The method of claim 1 where the said at least one compound is 1α-hydroxy-19-nor-24 epi-vitamin D_2.

9. The method of claim 1 where the said at least one compound is 1α,25-dihydroxy-19-nor-24 epi-vitamin D_2.

* * * * *

UNITED STATES PATENT AND TRADEMARK OFFICE

(12) CERTIFICATE EXTENDING PATENT TERM
UNDER 35 U.S.C. § 156

(68)	PATENT NO.	:	5,246,925
(45)	ISSUED	:	September 21, 1993
(75)	INVENTOR(S)	:	Hector F. DeLuca, et al.
(73)	PATENT OWNER	:	Wisconsin Alumni Research Foundation
(95)	PRODUCT	:	ZEMPLAR® (paricalcitol)

This is to certify that an application under 35 U.S.C. § 156 has been filed in the United States Patent and Trademark Office, requesting extension of the term of U.S. Patent No. 5,246,925 based upon the regulatory review of the product ZEMPLAR® (paricalcitol) by the Food and Drug Administration. Since it appears that the requirements of the law have been met, this certificate extends the term of the patent for the period of

(94) 574 days

from September 21, 2010, the original expiration date of the patent, subject to the payment of maintenance fees as provided by law, with all rights pertaining thereto as provided by 35 U.S.C. § 156(b).

I have caused the seal of the Patent and Trademark Office to be affixed this 18th day of July 2001.

Nicholas P. Godici

Nicholas P. Godici
Acting Under Secretary of Commerce for Intellectual Property and
Acting Director of the United States Patent and Trademark Office

UNITED STATES PATENT AND TRADEMARK OFFICE

(12) CERTIFICATE EXTENDING PATENT TERM
UNDER 35 U.S.C. § 156

(68)	PATENT NO.	:	5,246,925
(45)	ISSUED	:	September 21, 1993
(75)	INVENTOR(S)	:	Hector F. DeLuca, et al.
(73)	PATENT OWNER	:	Wisconsin Alumni Research Foundation
(95)	PRODUCT	:	ZEMPLAR® (paricalcitol)

This is to certify that an application under 35 U.S.C. § 156 has been filed in the United States Patent and Trademark Office, requesting extension of the term of U.S. Patent No. 5,246,925 based upon the regulatory review of the product ZEMPLAR® (paricalcitol) by the Food and Drug Administration. Since it appears that the requirements of the law have been met, this certificate extends the term of the patent for the period of

(94) 574 days

from September 21, 2010, the original expiration date of the patent, subject to the payment of maintenance fees as provided by law, with all rights pertaining thereto as provided by 35 U.S.C. § 156(b).

I have caused the seal of the Patent and Trademark Office to be affixed this 18th day of July 2001.

Nicholas P. Godici

Nicholas P. Godici
Acting Under Secretary of Commerce for Intellectual Property and
Acting Director of the United States Patent and Trademark Office

EXHIBIT B

US005587497A

United States Patent [19]

DeLuca et al.

[11] Patent Number: 5,587,497

[45] Date of Patent: Dec. 24, 1996

[54] **19-NOR-VITAMIN D COMPOUNDS**

[75] Inventors: **Hector F. DeLuca**, Deerfield; **Heinrich K. Schnoes**; **Kato L. Perlman**, both of Madison, all of Wis.; **Rafal R. Sicinski**, Warsaw, Poland; **Jean M. Prahl**, Madison, Wis.

[73] Assignee: **Wisconsin Alumni Research Foundation**, Madison, Wis.

[21] Appl. No.: **442,492**

[22] Filed: **May 16, 1995**

Related U.S. Application Data

[62] Division of Ser. No. 281,261, Jul. 27, 1994, abandoned, which is a division of Ser. No. 123,485, Sep. 17, 1993, Pat. No. 5,342,975, which is a division of Ser. No. 960,241, Oct. 13, 1992, Pat. No. 5,246,925, which is a continuation of Ser. No. 879,706, May 5, 1992, abandoned, which is a continuation of Ser. No. 557,400, Jul. 3, 1990, abandoned, which is a division of Ser. No. 481,354, Feb. 16, 1990, Pat. No. 5,237,110, which is a continuation-in-part of Ser. No. 321,030, Mar. 9, 1989, abandoned.

[51] **Int. Cl.**[6] **C07C 401/00**

[52] **U.S. Cl.** **552/653**

[58] **Field of Search** 552/653; 514/167

[56] **References Cited**

U.S. PATENT DOCUMENTS

4,410,515	10/1983	Holick et al.	424/180
4,448,726	5/1984	DeLuca et al.	260/397
4,769,181	9/1988	DeLuca et al.	260/397
5,086,191	2/1992	DeLuca et al.	552/653
5,185,150	2/1993	DeLuca et al.	424/195
5,246,925	9/1993	DeLuca et al.	514/167
5,281,731	1/1994	DeLuca et al.	552/653
5,321,018	6/1994	DeLuca et al.	514/617
5,342,975	8/1994	DeLuca et al.	552/653
5,403,940	4/1995	Valle's et al.	514/167
5,428,029	6/1995	Doran et al.	514/167
5,449,668	9/1995	Sestelo et al.	514/167
5,451,574	9/1995	Baggiolini et al.	514/167

FOREIGN PATENT DOCUMENTS

0250755	1/1988	European Pat. Off. .
WO85/03300	8/1985	WIPO .
WO86/02649	5/1986	WIPO .

OTHER PUBLICATIONS

Chemical Abstracts (110:19004r) 1989.

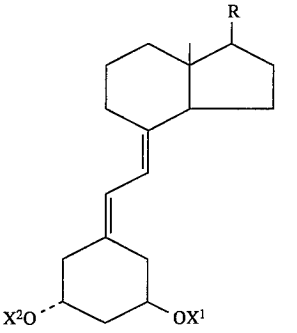
Lythgoe et al "Calciferol And Its Relatives. Part 22. A Direct Total Synthesis of Vitamin D3", Journal of The Chemical Society, Perkin Transactions I, vol. 6, 1978, pp. 590–595.

Primary Examiner—Kimberly J. Prior

Attorney, Agent, or Firm—Andrus, Sceales, Starke & Sawall

[57] **ABSTRACT**

This invention provides a novel class of vitamin D-related compounds, namely the 1α-hydroxy-19-nor-vitamin D analogs, as well as a general method for their chemical synthesis. The compounds exhibit pronounced activity in arresting the proliferation of undifferentiated cells, including malignant cells, and in inducing their differentiation, and thus represent novel therapeutic agents for the treatment of malignant and other diseases characterized by the proliferative growth of undifferentiated cells. Formulations for therapeutic use and treatment methods are also provided. The 19-nor vitamin D compounds have the formula:



where X^1 and X^2 are each hydrogen or a hydroxy protecting group and R is a side chain.

12 Claims, No Drawings

19-NOR-VITAMIN D COMPOUNDS

This invention was made with United States government support awarded by the Department of Health and Human Services (NIH), Grant number: DK-14881. The United States Government has certain rights in this invention.

This application is a division of application Ser. No. 08/281,261 filed Jul. 27, 1994, now abandoned, which in turn is a divisional of Ser. No. 08/123,485 filed Sep. 17, 1993, now U.S. Pat. No. 5,342,975, which in turn is a divisional of Ser. No. 07/960,241 filed Oct. 13, 1992, now U.S. Pat. No. 5,246,925, which in turn is a continuation of Ser. No. 07/879,706 filed May 5, 1992, now abandoned, which in turn is a continuation of Ser. No. 07/557,400 filed Jul. 23, 1990, now abandoned, which in turn is a divisional of Ser. No. 07/481,354 filed Feb. 16, 1990, now U.S. Pat. No. 5,237,110, which in turn is a continuation-in-part application of Ser. No. 07/321,030 filed Mar. 9, 1989, now abandoned.

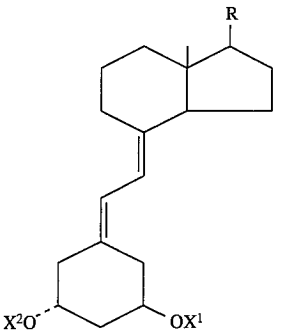
This invention relates to biologically active vitamin D compounds. More specifically, the invention relates to 19-nor-analogs of 1_{α}-hydroxylated vitamin D compounds and to a general process for their preparation.

BACKGROUND

The 1_{α}-hydroxylated metabolites of vitamin D—most importantly 1α,25-dihydroxyvitamin D_3 and 1α,25-dihydroxyvitamin D_2—are known as highly potent regulators of calcium homeostasis in animals and humans, and more recently their activity in cellular differentiation has also been established. As a consequence, many structural analogs of these metabolites, such as compounds with different side chain structures, different hydroxylation patterns, or different stereochemistry, have been prepared and tested. Important examples of such analogs are 1α-hydroxyvitamin D_3, 1α-hydroxyvi tamin D_2, various side chain fluorinated derivatives of 1α,25-dihydroxyvitamin D_3, and side chain homologated analogs. Several of these known compounds exhibit highly potent activity in vito or in vitro, and possess advantageous activity profiles and thus are in use, or have been proposed for use, in the treatment of a variety of diseases such as renal osteodystrophy, vitamin D-resistant rickets, osteoporosis, psoriasis, and certain malignancies.

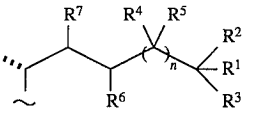
DISCLOSURE AND DESCRIPTION OF THE INVENTION

A class of 1α-hydroxylated vitamin D compounds not known heretofore are the 19-nor-analogs, i.e. compounds in which the ring A exocyclic methylene group (carbon 19) typical of all vitamin D system has been removed and replaced by two hydrogen atoms. Structurally these novel analogs are characterized by the general formula I shown below:



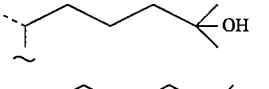
I

where X^1 and X^2 are each selected from the group consisting of hydrogen and acyl, and where the group R represents any of the typical side chains known for vitamin D type compounds. Thus, R may be an alkyl, hydrogen, hydroxyalkyl or fluoroalkyl group, or R may represent the following side chain:

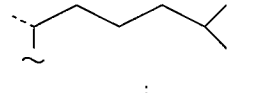


wherein R^1 represents hydrogen, hydroxy or O-acyl, R^2 and R^3 are each selected from the group consisting of alkyl, hydroxyalkyl and fluoroalkyl, or, when taken together represent the group $—(CH_2)_m—$where m is an integer having a value of from 2 to 5, R^4 is selected from the group consisting of hydrogen, hydroxy, fluorine, O-acyl, alkyl, hydroxyalkyl and fluoroalkyl, R^5 is selected from the group consisting of hydrogen, fluorine, alkyl hydroxyalkyl and fluoroalkyl, or, R^4 and R^5 taken together represent double-bonded oxygen, R^6 and R^7 are each selected from the group consisting of hydrogen, hydroxy, O-acyl, fluorine and alkyl, or, $R^6$and R^7 taken together form a carbon-carbon double bond, and wherein n is an integer having a value of from 1 to 5, and wherein the carbon at any one of positions 20, 22, or 23 in the side chain may be replaced by an O, S, or N atom.

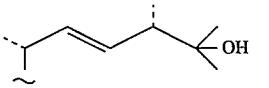
Specific important examples of side chains are the structures represented by formulas (a), (b), (c), (d) and (e) below, i.e. the side chain as it occurs in 25-hydroxyvitamin D_3 (a); vitamin D_3 (b); 25-hydroxyvitamin D_2 (c) ; vitamin D_2 (d) ; and the C-24-epimer of 25-hydroxyvitamin D_2 (e).



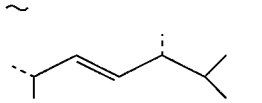
(a)



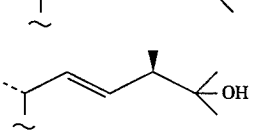
(b)



(c)



(d)

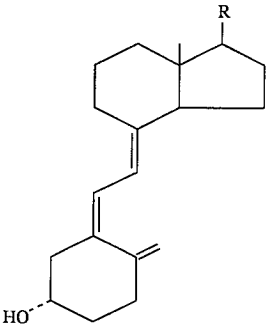


(e)

In this specification and the claims, the term 'alkyl' signifies an alkyl radical of 1 to 5 carbons in all isomeric forms, such as methyl, ethyl, propyl, isopropyl, butyl, isobutyl, pentyl, etc., and the terms 'hydroxyalkyl' and 'fluoro-

alkyl' refer to such an alkyl radical substituted by one or more hydroxy or fluoro groups respectively, and the term 'acyl' means an aliphatic acyl group of 1 to 5 carbons, such as formyl, acetyl, propionyl, etc. or an aromatic acyl group such as benzoyl, nitrobenzoyl or halobenzoyl. The term 'aryl' signifies a phenyl-, or an alkyl-, nitro- or halo-substituted phenyl group.

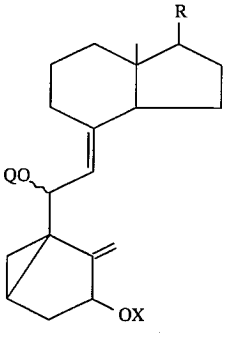
The preparation of 1_{α}-hydroxy-19-nor-vitamin D compounds having the basic structure shown above can be accomplished by a common general method, using known vitamin D compounds as starting materials. Suitable starting materials are, for example, the vitamin D compounds of the general structure II:



II

where R is any of the side chains as defined above. These vitamin D starting materials are known compounds, or compounds that can be prepared by known methods.

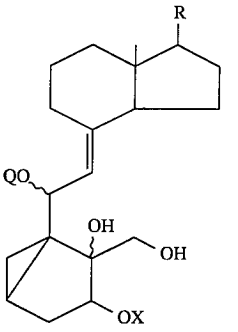
Using the procedure of DeLuca et al. (U.S. Pat. No. 4,195,027), the starting material is converted to the corresponding 1α-hydroxy-3,5-cyclovitamin D derivative, having the general structure III below, where X represents hydrogen and Q represents an alkyl, preferably methyl:



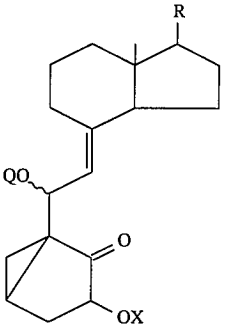
III

So as to preclude undesired reaction of the 1α-hydroxy group in subsequent steps, the hydroxy group is converted to the corresponding acyl derivative, i.e. the compound III shown above, where X represents an acyl group, using standard acylation procedures, such as treatment with an acyl anhydride or acyl halide- in pyridine at room temperature or slightly elevated temperature (30°–70° C.). It should be understood also that whereas the process of this invention is illustrated here with acyl protection of hydroxy functions, alternative standard hydroxy-protecting groups can also be used, such as, for example, alkylsilyl or alkoxyalkyl groups. Such protecting groups are well-known in the art. (e.g. trimethylsilyl, triethylsilyl, t.-butyldimethylsilyl, or tetrahydrofuranyl methorymethyl), and their use is considered a routine modification of experimental detail within the scope of the process of this invention.

The derivative as obtained above is then reacted with osmium tetroxide, to produce the 10,19-dihydroxy analog, IV (where X is acyl), which is subjected to diol cleavage using sodium metaperiodate or similar vicinal diol cleavage reagents (e.g. lead tetraacetate) to obtain the 10-oxo-intermediate, having the structure V below (where X is acyl):



IV

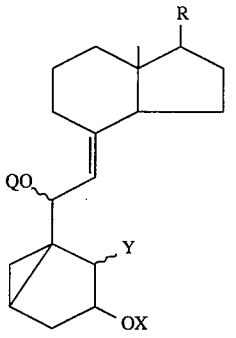


V

These two consecutive steps can be carried out according to the procedures given by Paaren et el. [J. Org. Chem. 48, 3819 (1983)]. If the side chain unit, R, carries vicinal diols (e.g. 24,25-dihydroxy-or 25,26-dihydroxy, etc.), these, of course, also need to be protected, e.g. via acylation, silylation, or as the isopropylidene derivative prior to the periodate cleavage reactions.

In most cases, the acylation of the 1α-hydroxy group as mentioned above will simultaneously effect the acylation of side chain hydroxy functions, and these acylation conditions can, of course, be appropriately adjusted (e.g. elevated temperatures, longer reaction times) so as to assure complete protection of side chain vicinal diol groupings.

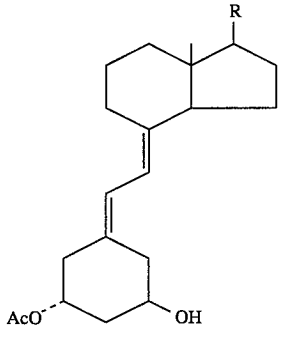
The next step of the process comprises the reduction of the 10-oxo-group to the corresponding 10-alcohol having the structure VI shown below (where X is acyl and Y represents hydroxy). When X is acyl, this reduction is carried out conveniently in an organic solvent at from about 0° C. to about room temperature, using $NaBH_4$ or equivalent hydride reducing agents, selective for the reduction of carbonyl groups without cleaving ester functions. Obviously, when X is a hydroxy-protecting group that is stable to reducing agents, any of the other hydride reducing agents (e.g. $LiAlH_4$, or analogous reagents) may be employed also.



VI

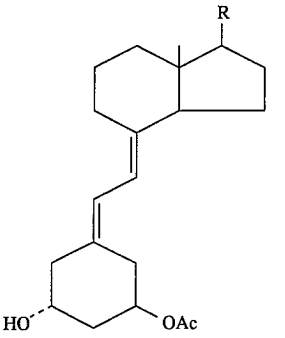
The 10-hydroxy intermediate is then treated with an alkyl- or arylsulfonylhalide (e.g. mathanesulfonylchloride) in a suitable solvent (e.g. pyridine) to obtain the corresponding 10-O-alkyl—or arylsulfonyl derivative (the compound having the structure shown VI above where Y is alkyl-SO_2O-, or aryl-SO_2O-, and this sulfonate intermediate is then directly reduced, with lithiun aluminum hydride, or the analogous known lithium aluminum alkyl hydride reagents in an ether solvent, at a temperature ranging from 0° C. to the boiling temperature of the solvent, thereby displacing the sulfonate group and obtaining the 10-deoxy derivative, represented by the structure VI above, where X and Y are both hydrogen. As shown by the above structure, a 1-O-acyl function in the precursor compound V is also cleaved in this reduction step to produce the free 1α-hydroxy function, and any O-acyl protecting group in the side chain would, of course, likewise be reduced to the corresponding free alcohol function, as is well understood in the art. If desired, the hydroxy groups at C-1 (or hydroxy groups in the side chain) can be reprotected by acylation or silylation or ether formation to the corresponding acyl, alkylsilyl or alkoxyalkyl derivative, but such protection is not required. Alternative hydroxy-protecting groups, such as alkylsilyl or alkoxyalkyl groups would be retained in this reduction step, but can be removed, as desired, at this or later stages in the process by standard methods known in the art.

The above 1α-hydroxy-10-deoxy cyclovitamin D intermediate is next solvolyzed in the presence of a low-molecular weight organic acid, using the conditions of DeLuca et al. (U.S. Pat. Nos. 4,195,027 and 4,260,549). When the solvolysis is carried out in acetic acid, for example, there is obtained a mixture of 1α-hydrox-19-nor-vitamin D 3-acetate and 1α-hydroxy-19-nor-vitamin D 1-acetate (compounds VII and VIII, below), and the analogous 1- and 3-acylates are produced, when alternative acids are used for solvolysis.



VII

-continued



VIII

Direct basic hydrolysis of this mixture under standard conditions then produces the desired 1α-hydroxy-19-nor-vitamin D compounds of structure I above (where X^1 and X^2 are hydrogen). Alternatively, the above mixture of monacetates may also be separated (e.g. by high pressure liquid chromatography) and the resulting 1-acetate and 3-acetate isomers may be subjected separately to hydrolysis to obtain the same final product from each, namely the 1α-hydroxy-19-nor-vitamin D compounds of structure I. Also the separated monoacetates of structure VII or VIII or the free 1,3-dihydroxy compound can, of course, be reacylated according to standard procedures with any desired acyl group, so as to produce the product of structure I above where X^1 and X^2 represent acyl groups which may be the same or different.

Biological Activity of 1α-Hydroxy-19-Nor-Vitamin D-Vitamin D Compounds

The novel compounds of this invention exhibit an unexpected parttern of biological activity, namely high potency in promoting the differentiation of malignant cells and little or no activity in calcifying bone tissue. This is illustrated by the biological assay results obtained for 1α,25-dihydroxy-19-nor-vitamin D_3 (compounds Ia), which are summarized in Tables 1 and 2, respectively. Table 1 shows a comparison of the activity of the known active metabolite 1α,25-dihydroxyvitamin D_3 and the 19-nor analog (Ia) in inducing the differentiation of human leukemia cells (HL-60 cells) in culture to normal cells (monocytes). Differentiation activity was assessed by three standard differentiation assays, abbreviated in Table 1 as NBT (nitroblue tetrazolium reduction), NSE (non-specific esterase activity), and PHAGO (phagocytosis activity). The assays were conducted according to known procedures, as given, for example, by DeLuca et el. (U.S. Pat. No. 4,717,721) and Ostrem et el., J. Biol. Chem. 262, 1416, 1987). For each assay, the differentiation activity of the test compounds is expressed in terms of the percent of HL-60 cells having differentiated to normal cells in response to a given concentration of test compound.

The results summarized in Table 1 clearly show that the new analog, 1α,25-dihydrory-19-nor-vitamin D_3 (Ia) is as potent as 1α,25-dihydroxyvitamin D_3 in promoting the differentiation of leukemia cells. Thus in all three assays close to 90% of the cells are induced to differentiate by 1α,25-dihdyroxy-vitamin D_3 at a concentration of 1×10^{-7} molar, and the same degree of differentiation (i.e. 90, 84 and 90%) is achieved by the 19-nor analog (Ia).

TABLE 1

Differentiation of HL-60 Cells			
	% Differentiated Cells (mean ± SEM)		
	NBT	NSE	PRAGO
1α,25-dihydroxyvitamin D_3 (moles/liter)			
1×10^{-7}	86 ± 2	89 ± 1	87 ± 3
1×10^{-8}	60 ± 2	60 ± 3	64 ± 2
1×10^{-9}	33 ± 2	31 ± 2	34 ± 1
1α,25-Dihydroxy-19-nor-vitamin D_3, (Ia) (moles/liter)			
2×10^{-7}	94 ± 2	95 ± 3	94 ± 2
1×10^{-7}	90 ± 4	84 ± 4	90 ± 4
5×10^{-8}	72 ± 3	73 ± 3	74 ± 3
1×10^{-8}	61 ± 3	60 ± 3	56 ± 1
1×10^{-9}	32 ± 1	31 ± 1	33 ± 1

In contrast to the preceding results, the new 19-nor analog (Ia) exhibits no activity in an assay measuring the calcification of bone, a typical response elicited by vitamin D compounds. Relevant data, representing the results of an assay comparing the bone calcification activity in rats of 1α,25-dihydroxyvitamin D_3 and 1α,25-dihydroxy-19-nor-vitami D_3 (Ia), are summarized in Table 2. This assay was conducted according to the procedure described by Tanaka et al., Endocrinology 92, 417 (1973).

The results presented in Table 2 show the expected bone calcification activity of 1α,25-dihydroxyvitamin D_3 as reflected by-the increase in percent bone ash, and in total ash at all dose levels. In contrast, the 19-nor analog Is exhibits no activity at all three dose levels, when compared to the vitamin D-deficient (-D) control group.

TABLE 2

Calcification Activity			
Compound	Amount Administered* (pmoles/day/7 days)	% Ash (mean ± SEM)	Total Ash (mg) (mean ± SEM)
-D (control)	0	19 ± 0.8	23 ± 1.2
1α,25-dihydroxy-vitamin D_3	32.5	23 ± 0.5	34 ± 1.6
	65.0	26 ± 0.7	36 ± 1.1
	325.0	28 ± 0.9	40 ± 1.9
1α,25-dihydroxy-19-nor-vitamin D_3 (Ia)	32.5	22 ± 0.9	28 ± 1.6
	65.0	19 ± 1.5	28 ± 3.4
	325.0	19 ± 1.2	30 ± 2.4

*Each assay group comprised 6 rats, receiving the indicated amount of test compound by intraperitoneal injection daily for a period of seven days.

Thus the new 19-nor analog shows a selective activity profile combining high potency in inducing the differentiation of malignant cells with very low or no bone calcification activity. The compounds of this novel structural class, therefore, can be useful as therapeutic agents for the treatment of malignancies Because the differentiative activity of vitamin D compounds on keratinocytes of skin (Smith et el., J. Invest. Dermatol. 86, 709, 1986; Smith et el., J. Am. Aced. Dermatol. 19, 516, 1988) is believed to be an indication of successful treatment of psoriasis (Takamoto et al., Calc. Tissue Int. 39, 360, 1986), these compounds should prove useful in treating this and other skin disorders characterized by proliferation of undifferentiated skin cells. These compounds should also find use in the suppression of parathyroid tissue, as for example, in cases of secondary hyperparathyroidism found in renal disease (Slatopolsky et al., J. Clin. Invest. 74, 2136, 1984).

For treatment purposes, the novel compounds of this invention can be formulated as solutions in innocuous solvents, or as emulsions, suspensions or dispersions in suitable innocuous solvents or carriers, or as pills, tablets or capsules, containing solid carriers according to conventional methods known in the art. For topical applications the compounds are advantageously formulated as creams or ointments or similar vehicle suitable for topical applications. Any such formulations may also contain other pharmaceutically-acceptable and non-toxic excipients such as stabilizers, anti-oxidants, binders, coloring agents or emulsifying or taste-modifying agents.

The compounds are advantageously administered by injection, or by intravenous infusion of suitable sterile solutions, or in the form of oral doses via the alimentary canal, or topically in the form of ointments, lotions, or in suitable transdermal patches. For the treatment of malignant diseases, the 19-nor-vitamin D compounds of this invention are administered to subjects in dosages sufficient to inhibit the proliferation of malignant cells and induce their differentiation into normal monocyte-macrophages. Similarly, for the treatment of psoriasis, the compounds may be administered orally or topically in amounts sufficient to arrest the proliferation of undifferentiated keratinocytes, and in the treatment of hyperparathyroidism, the compounds are administered in dosages sufficient to suppress parsthyroid activity, so as to achieve parathyroid hormone levels in the normal range. Suitable dosage amounts are from 1 to 500 μg of compound per day, such dosages being adjusted, depending on diseases to be treated, its severity and the response or condition of the subject as well-understood in the art.

This invention is more specifically described by the following illustrative examples. In these examples specific products identified by Roman numerals and letters, i.e. Ia, Ib, . . . , IIa, IIb, . . . , etc. refer to the specific structures and side chain combinations identified in the preceding description.

EXAMPLE 1

Preparation of 1α,25-dihydroxy-19-nor-vitamin D_3 (Ia)

(a) 1α,25-Dihydroxy-3,5-cyclovitamin D_3 1-acetate, 6-methyl ether.: Using 25-hydroxyvitamin D_3 (IIa) as starting material, the known 1α,25-dihydroxy-3,5-cyclovitamin D_3 derivative IIIa (X=H) was prepared according to published procedures (DeLuca et al., U.S. Pat. No. 4,195,027 and Paaren et al., J. Org. Chem. 45, 3252 (1980)). This product was then acetylated under standard conditions to obtain the corresponding 1-acetate derivative IIIa (X≈Ac).

(b) 10,19-Dihydro-1α,25-tetrahydroxy-3,5-cyclovitamin D_3 1-acetate, 6-methyl ether (IVa): Intermediate IIIa (X=Ac) was treated with a slight molar excess of osmium tetroxide in pyridine according to the general procedure described by Paaren et el. (J. Org. Chem. 48, 3819 (1983)) to obtain the 10,19-dihydroxylated derivative IVa. Mass spectrum m/z (relative intensity), 506 (M^+, 1) 488 (2) 474 (40) 425 (45), 396 (15), 285 (5), 229 (30), 133 (45), 59 (80), 43 (100). ^1H NMR ($CDCl_3$) δ 0.5 (3H, s, 18-CH_3), 0.58 (1H, m, 3-H), 0.93 (3H, d, J=6.1 Hz, 21-CH_3), 1.22 (6H, s, 26-CH_3 and 27-CH_3), 2.10 (3H, s, $COCH_3$), 3.25 (3H, s, 6-OCR_3), 3.63 (2H, m, 19-CH_2), 4.60 (1H, d, J=9.2 Hz, 6-H), 4.63 (1H, dd, 1β-H), 4.78 (1H, d, J=9.2 Hz, 7-H).

(c) 1α,25-Dihydroxy-10-oxo-3,5-cyclo-19-nor-vitamin D_3 1-acetate, 6-methyl ether (Va): The 10,19-dihydroxylated

intermediate IVa was treated with a solution of sodium metapariodate according to the procedure given by Paaren et al. (J. Org. Chem. 48, 3819, 1983) to produce the 10-oxo-cyclovitamin D derivative (Va, X=Ac). Mass spectrum m/z (relative intensity) 442 (M^+-MeOH) (18), 424 (8), 382 (15), 364 (35), 253 (55), 225 (25), 197 (53), 155 (85), 137 (100). ^1H NMR ($CDCl_3$) δ 0.58 (3H, s, 18-CH_3), 0.93 (3H, d, J=6.6 Hz, 21-CH_3), 1.22 (6H, s, 26-CH_3 and 27-CH_3), 2.15 (s, 3-$OCOCH_3$), 3.30 (3H, s, 6-OCH_3), 4.61 (1H, d, J=9.1Hz, 6-H), 4.71 (1H, d, J=9.6 Hz, 7-H), 5.18 (1H, m, 1β-H).

It has been found also that this diol cleavage reaction does not require elevated temperatures, and it is, indeed, generally prefereable to conduct the reaction at approximately room temperature.

(d) 1α,-Acetoxy-10,25-dihydroxy-3,5-cyclo-19-nor-vitamin D_3 6-methyl ether (VIa, X=Ac, Y=OH): The 10-oxo derivative Va (X=Ac) (2.2 mg, 4.6 μmol) was dissolved in 0.5 ml of ethanol and to this solution 50 μl (5.3 μmol) of a $NaBH_4$ solution (prepared from 20 mg of $NaBH_4$, 4.5 ml water and 0.5 ml of 0.0 N NaOH solution) was added and the mixture stirred at 0° C. ca. 1.5 h, and then kept at 0° C. for 16 h. To the mixture ether yes added and the organic phase washed with brine, dried over $MgSO_4$, filtered and evaporated. The crude product was purified by column chromatography on a 15×1 cm silica gel column and the alcohol Via (X=Ac, Y=OH) was eluted with ethyl acetate hexane mixtures to give 1.4 mg (3 μmol) of product. Mass spectrum m/z (relative intensity) 476 (M^+) (1), 444 (85), 426 (18), 384 (30), 366 (48), 351 (21), 255 (35), 237 (48), 199 (100), 139 (51),59 (58).

(e) 1α,25-Dihydroxy-19-nor-vitamin D_3 (Ia, X^1=X^2=H): The 10-alcohol (VIa, X=Ac, Y=OH) (1.4 mg) was dissolved in 100 μl anhydrous CH_2Cl_2 and 10 μl (14 μmol) triethylamine solution [prepared from 12 mg (16 μl) triethylamine in 100 μl anhydrous CH_2Cl_2], followed by 7 μl (5.6 μmol) mesyl chloride solution (9 mg mesyl chloride, 6.1 μl, in 100 μl anhydrous CH_2Cl_2) added at 0° C. The mixture was stirred at 0° C. for 2 h. The solvents were removed with a stream of argon and the residue (comprising compound VIa, X=Ac, Y=CH_3SO_2O-) dissolved in 0.5 ml of anhydrous tetrahydrofuran; 5 mg of $LiAlH_4$ was added at 0° C. and the mixture kept at 0° C. for 16 h. Excess $LiAlH_4$ was decomposed with wet ether, the ether phase was washed with water and dried over $MgSO_4$, filtered and evaporated to give the 19-nor product VIa (X=Y=H).

This product was dissolved in 0.5 ml of acetic acid and stirred at 55° C. for 20 min. The mixture was cooled, ice water added and extracted with ether. The other phase was washed with cold 10% sodium bicarbonate solution, brine, dried over $MgSO_4$, filtered and evaporated to give the expected mixture of 3-acetoxy-1α-hydroxy- and 1α-acetoxy-3-hydroxy isomers, which were separated and purified by HPLC (Zorbax Sil column, 6.4×25 cm, 2-propanol in hexane) to give about 70 μg each of compounds VIIa and XIIIa. UV (in EtOH) λ_{max} 242.5 (OD 0.72), 251.5 (OD 0.86), 260 (OD 0.57).

Both 19-nor-1,25-dihydroxyvitamin D_3 acetates VIIa and VIIIa were hydrolyzed in the same manner. Each of the monoacetates was dissolved in 0.5 ml of ether and 0.5 ml 0.1 N KOH in methanol was added. The mixture was stirred under argon atmosphere for 2 h. More ether was added and the organic phase washed with brine, dried over anhydrous $MgSO_4$, filtered and evaporated. The residue was dissolved in a 1:1 mixture of 2-propanol and hexane and passed through a Sep Pak column and washed with the same solvent. The solvents were evaporated and the residue purified by HPLC (Zorbax Sil, 6.4×25 cm, 10% 2-propanol in hexane). The hydrolysis products of VIIa and VIIIa were identical and gave 66 μg of Ia (X^1=X^2=H). Mass spectrum (m/z relative intensity) 404 (M^+) (100), 386 (41), 371 (20), 275 (53), 245 (51), 180 (43), 135 (72), 133 (72), 95 (82), 59 (18), exact mass calcd. for $C_{26}H_{44}O_3$ 404.3290, found 404.3272. ^1H NMR ($CDCl_3$) δ 0.52 (3H, s, 18-CH_3), 0.92 (3H, d, J=6.9 Hz, 21-CH_3), 1.21 (6H, s, 26-CH_3 and 27-CH_3), 4.02 (1H, m, 3α-H), 4.06 (1H, m, 1β-H), 5.83 (1H, d, J=11.6 Hz, 7-H), 6.29 (1H, d, J=10.7 Hz, 6-H). UV (in EtOH) λ_{max} 243 (OD 0.725), 251.5 (OD 0.823), 261 (OD 0.598).

EXAMPLE 2

Preparation of 1α-hydrory-19-nor-vitamin D_3 (Ib)

(a) With vitamin D_3 (IIb) as starting material, and utilizing the conditions of Example 1a, There is obtained known 1α-hydroxy-3,5-cyclovitamin D_3 1-acetate, 6-methyl ether, compound IIIb (X=Ac).

(b) By subjecting intermediate IIIb (X=Ac), as obtained in Example 2a above to the conditions of Example 1b, there is obtained 10,19-dihydro-1α,10,19-trihydroxy-3,5-cyctovitamin D_3 1-acetate, 6-methyl ether IVb (X=Ac).

(c) By treatment of intermediate IVb (X=Ac) with sodium metaperiodate according to Example 1c above, there is obtained 1α-hydroxy-10-oxo-3,5-cyclo-19-nor-vitamin D_3 1-acetate, 6-methyl ether Vb (X=Ac).

(d) Upon reduction of the 10-oxo-intermediate Vb (X=Ac) under the conditions of Example 1d above, there is obtained 1α-acetoxy-10-hydroxy-3,5-cyclo-19-nor-vitamin D_3 6-methyl ether VIb (X=Ac, Y=OH).

(e) Upon processing intermediate VIb (X=Ac, Y=OH) through the procedure given in Example 1e above, there is obtained 1α-hydroxy-19-nor-vitamin D_3 (Ib, X^1=X^2=H).

EXAMPLE 3

Preparation of 1α,25-dihydroxy-19-nor-vitamin D_2

(a) Utilizing 25-hydroxyvitamin D_2 (IIc) as starting material and experimental conditions analogous to those of Example 1a, there is obtained 1α,25-dihydroxy-3,5-cyclovitamin D_2 1-acetate, 6-methyl ether, compound IIIc (X=Ac).

(b) Subjecting intermediate IIId (X=Ac), as obtained in Example 3a above, to the reaction conditions of Example Ib, provides 10,19-dihydro-1α,10,19,25-tetrahydroxy-3,5-cyclo-vitamin D_2 1-acetate, 6-methyl ether, IVc (X=Ac).

(c) By treatment of intermediate IVc (X=Ac) with sodium metaperiodate according to general procedures of Example 1c above, there is obtained 1α,25-dihydroxy-10-oxo-3,5-cyclo-19-nor-vitamin D_2 1-acetate, 6-methyl ether Vc (X=Ac).

(d) Upon reduction of the 10-oxo-intermediate Vc (X=Ac) under conditions analogous to those of Example 1d above, there is obtained 1α-acetoxy-10,25-dihydroxy-3,5-cyclo-19-nor-vitamin D_2 6-methyl ether VIc (X=Ac, Y=OH).

(e) Upon processing intermediate VIc (X=Ac, Y=OH) through the procedural steps given in Example 1e above, there is obtained 1α,25-dihydroxy-19-nor-vitamin D_2 (Ic, X^1=X^2=H).

EXAMPLE 4

Preparation of 1α-hydroxy-19-nor-vitamin D_2

(a) With vitamin D_2 (IId) as starting material, and utilizing the conditions of Example 1a, there is obtained known 1α-hydroxy-3,5-cyclovitamin D_2 1-acetate, 6-methyl ether, compound IIId (X=Ac).

(b) By subjecting intermediate IIId (X=Ac), as obtained in Example 4a above to the conditions of Example 1b, there is obtained 10,19-dihydro-1α,10,19-trihydroxy-3,5-cyclovitamin D_2 1-acetate, 6-methyl ether, IVd (X=Ac).

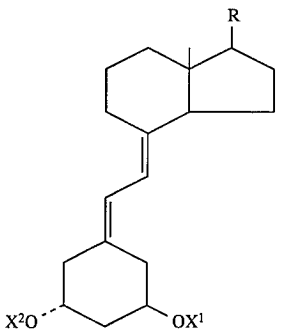
(c) By treatment of intermediate IVb (X=Ac) with sodium metaperiodate according to Example 1c above, there is obtained 1α-hydroxy-10-oxo-3,5-cyclo-19-nor-vitamin D_2 1-acetate, 6-methyl ether, Vd (X=Ac).

(d) Upon reduction of the 10-oxo-intermediate Vd (X=Ac) under the conditions of Example 1d above, there is obtained 1α-acetory-10-hydroxy-3,5-cyclo-19-nor-vitamin D_2 6-methyl ether, VId (X=Ac, Y=OH).

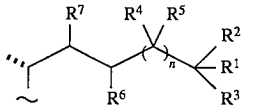
(e) Upon processing intermediate VId (X=Ac, Y=OH) through the procedure given in Example 1e above, there is obtained 1α-hydroxy-19-nor-vitamin D_2 (Id, X^1=X^2=H).

We claim:

1. A compound having the formula



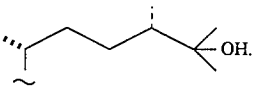
where X^1 and X^2 are each selected from hydrogen, acyl, alkylsilyl and alkoxyalkyl, and where R is selected from alkyl, hydrogen, hydroxyalkyl, said hydroxyalkyl having from 4 to 5 carbon atoms, fluoroalkyl and a side chain of the formula



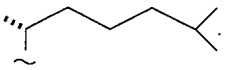
wherein R^1 represents hydrogen, hydroxy or O-acyl, R^2 and R^3 are each selected from alkyl, hydroxyalkyl and fluoroalkyl, or, when taken together represent the group $—(CH_2)_m—$where m is an integer having a value of from 2 to 5, R^4 is selected from hydrogen, hydroxy, fluorine, O-acyl, alkyl, hydroxyalkyl and fluoroalkyl, R^5 is selected from hydrogen, fluorine, alkyl, hydroxyalkyl and fluoroalkyl, or R^4 and R^5 taken together represent double-bonded oxygen, R^6 and R^7 are each selected from hydrogen, hydroxy, O-acyl, fluorine and alkyl, or, R^6 and R^7 taken together form a carbon-carbon double bond, and wherein n is an integer having a value of from 1 to 5 and wherein any of the groups -$CH(CH_3)$-, -$CH(R^7)$-, or -$CH(R^6)$- at positions 20, 22 and 23, respectively, may be replaced by an oxygen atom, with the proviso that when n is 2 to 5 each R^4 is independently selected from hydrogen, hydroxy, fluorine, O-acyl, alkyl, hydroxyalkyl and fluoroalkyl, and each R^5 is independently selected from hydrogen, fluorine, alkyl, hydroxyalkyl and fluoroalkyl.

2. A compound according to claim 1 where X^1 and X^2 represent hydrogen, and where R^1 is hydroxy, both of R^2 and R^3 are selected from the group consisting of methyl, trifluoromethyl, ethyl and propyl, both of R^6 and R^7 are hydrogen, or together form a carbon-carbon double bond, R^4 and R^5 are hydrogen and n is an integer having the values 1, 2 or 3.

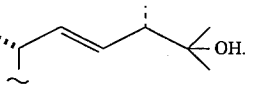
3. The compound of claim 1 where R is a side chain of the formula



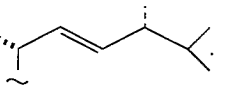
4. The compound of claim 1 where R is a side chain of the formula



5. The compound of claim 1 where R is a side chain of the formula



6. The compound of claim 1 where R is a side chain of the formula



7. 1α,25-dihydroxy-19-nor-vitamin D_3.

8. 1α-hydroxy-19-nor-vitamin D_3.

9. 1α,25-dihydroxy-19-nor-vitamin D_2 .

10. 1α-hydroxy-19-nor-vitamin D_2.

11. 1α-hydroxy-19-nor-24 epi-vitamin D_2.

12. 1α,25-dihydroxy-19-nor-24 epi-vitamin D_2.

* * * * *

EXHIBIT C

US006136799A

United States Patent [19]

Li et al.

[11] **Patent Number: 6,136,799**

[45] **Date of Patent: Oct. 24, 2000**

[54] **COSOLVENT FORMULATIONS**

[75] Inventors: **Lukchiu Li**, Vernon Hills; **Edward Anthony Pec**, Brookfield; **Daniel H. Robinson**, Lake Bluff; **Dennis A. Stephens**, Mt. Prospect, all of Ill.; **Kathee Jantzi**, Madison, Wis.; **Thomas Barton May**, Grayslake; **John Paul Oberdier**, Gurnee, both of Ill.

[73] Assignee: **Abbott Laboratories**, Abbott Park, Ill.

[21] Appl. No.: **09/057,143**

[22] Filed: **Apr. 8, 1998**

[51] **Int. Cl.**[7] **A61K 31/59**; A01N 45/00

[52] **U.S. Cl.** **514/167**; 424/236

[58] **Field of Search** 514/212, 424, 514/167; 424/236

[56] **References Cited**

U.S. PATENT DOCUMENTS

4,308,264	12/1981	Udipi	525/359.2
4,594,340	6/1986	Partridge et al.	514/167
4,855,294	8/1989	Patel et al.	514/212
5,098,899	3/1992	Gilbert et al.	514/167
5,502,224	3/1996	Eugster et al.	552/653
5,597,815	1/1997	Deluca et al.	514/167
5,629,302	5/1997	Eugster et al.	514/167

FOREIGN PATENT DOCUMENTS

0416137	3/1991	European Pat. Off. .	
0480572	4/1992	European Pat. Off. .	
2260903	5/1993	United Kingdom .	
9300079	1/1993	WIPO .	
9636340	11/1996	WIPO .	
97/37637	10/1997	WIPO	A61K 9/127

OTHER PUBLICATIONS

Bertino et al., Stability of an extemporaneous formulation . . . , American J. of Hosp. Pharmacy, V38(12), p. 1932–1933, 1981.

mortensen et al., Toxicity of 1,25–dihydroxyvitamin D3 . . . , Journal of Investigative Dermat . . . , V 1(1), p. 60–63, 1996.

Whittle et al., Intravenous vitamin D in the detection of . . . , The Lancet, vol. 1, pp. 747–750, (1969).

Bertino et al., Stability of an extra . . . , American J. of Hosp. Pharmacy, vol. 38/12, pp. 1932–1933, (1981).

Primary Examiner—Marianne M. Cintins

Assistant Examiner—Vickie Kim

Attorney, Agent, or Firm—Gregory W. Steele

[57] **ABSTRACT**

Stable pharmaceutical formulations of a therapeutic agent, a low molecular weight alcohol and a glycol derivative are disclosed. Preferred formulations include 19-nor-1α,3β,25-trihydroxy-9,10-secoergosta-5,7(E),22(E)-triene.

14 Claims, 3 Drawing Sheets

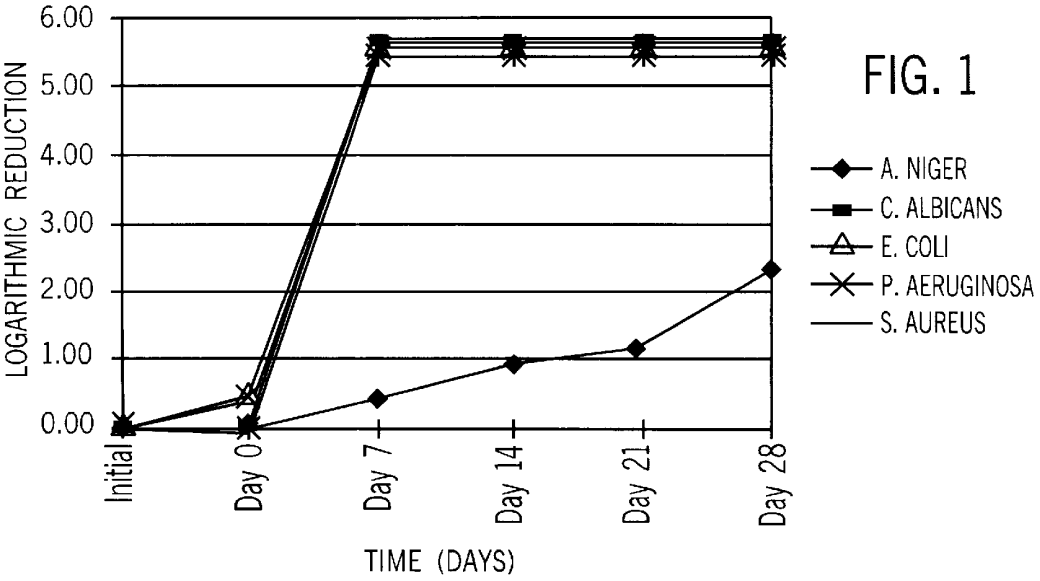

6.00
5.00
4.00
3.00
2.00
1.00
0.00
LOGARITHMIC REDUCTION
Initial
Day 0
Day 7
Day 14
Day 21
Day 28
TIME (DAYS)
A. NIGER
C. ALBICANS
E. COLI
P. AERUGINOSA
S. AUREUS

FIG. 1

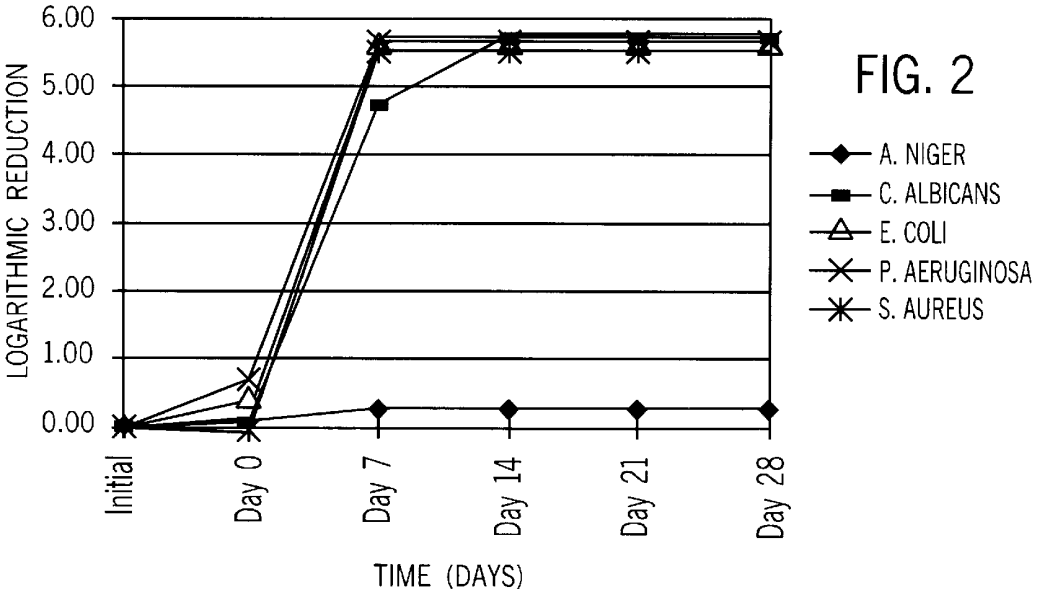

6.00
5.00
4.00
3.00
2.00
1.00
0.00
LOGARITHMIC REDUCTION
Initial
Day 0
Day 7
Day 14
Day 21
Day 28
TIME (DAYS)
A. NIGER
C. ALBICANS
E. COLI
P. AERUGINOSA
S. AUREUS

FIG. 2

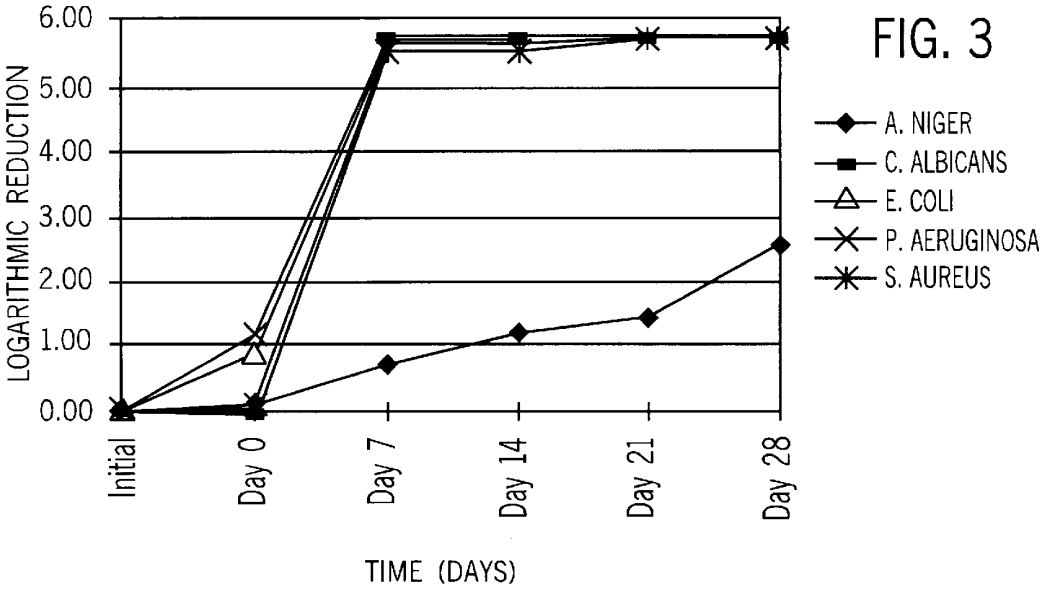


FIG. 3

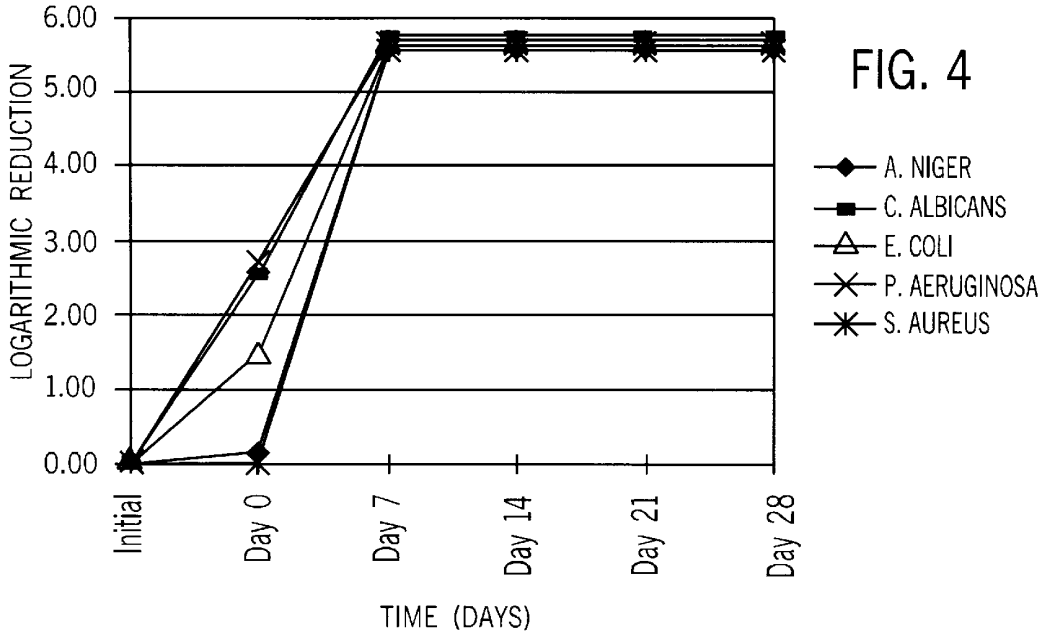


FIG. 4

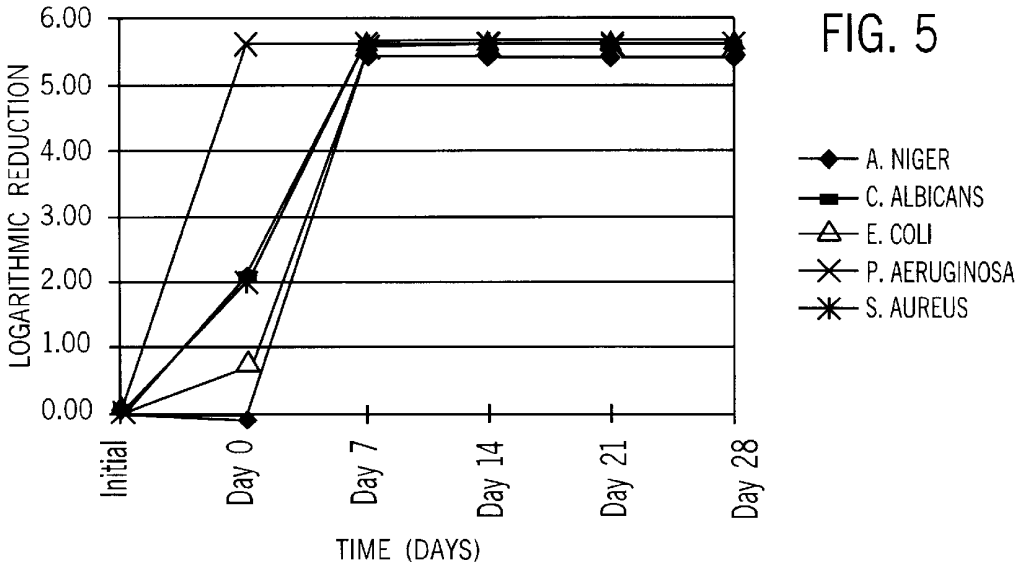

6.00
5.00
4.00
3.00
2.00
1.00
0.00
LOGARITHMIC REDUCTION
Initial
Day 0
Day 7
Day 14
Day 21
Day 28
TIME (DAYS)
A. NIGER
C. ALBICANS
E. COLI
P. AERUGINOSA
S. AUREUS

FIG. 5

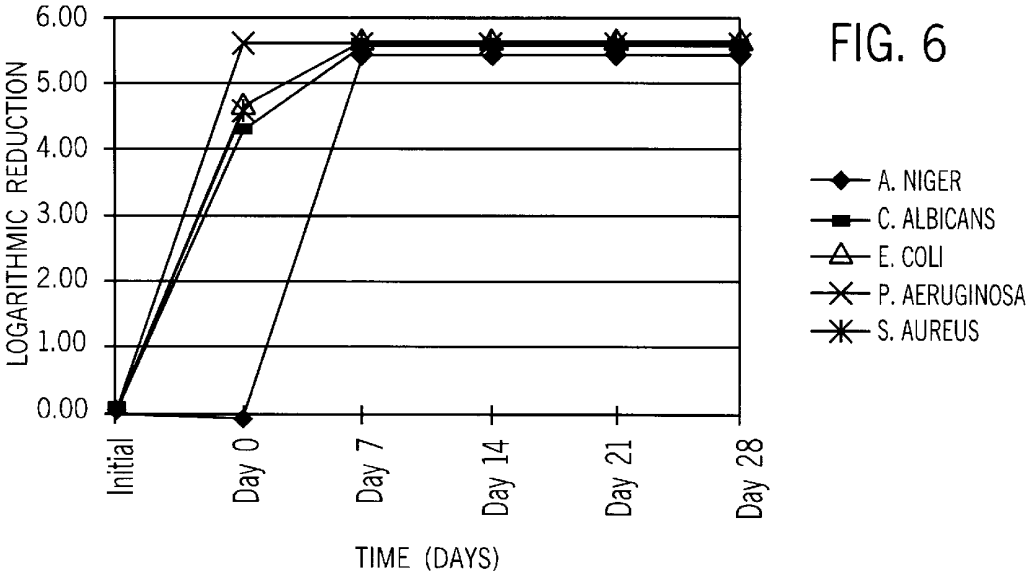

6.00
5.00
4.00
3.00
2.00
1.00
0.00
LOGARITHMIC REDUCTION
Initial
Day 0
Day 7
Day 14
Day 21
Day 28
TIME (DAYS)
A. NIGER
C. ALBICANS
E. COLI
P. AERUGINOSA
S. AUREUS

FIG. 6

COSOLVENT FORMULATIONS

BACKGROUND OF THE INVENTION

1. Field of the Invention

This invention relates to cosolvent formulations for therapeutic agents, the formulations having a synergistic preservative effect.

2. Discussion of the Prior Art

There is a continuing need to develop efficacious formulations for therapeutic agents that offer advantages in manufacturing, processing and safety for the patient. In particular, many therapeutic agents, for example vitamin D compounds, are oxygen sensitive or are otherwise unstable. Thus, the need to protect such compounds has lead to the routine addition of antioxidants in order to preserve the integrity of the active agent. In other formulations, buffers may be necessary to maintain pH. Chelating agents, including but not limited to citric acid, tartaric acid, amino acids, thioglycolic acid, and edetate disodium (EDTA), and buffers, including but not limited to acetate, citrate, glutamate, and phosphate buffers, are often used to stabilize formulations. However, as discussed in WO 96/36340, buffers and chelating agents have been implicated in imparting aluminum levels in products to in excess of 3.5 parts per million at the expiration date of the product.

It would be particularly advantageous to minimize aluminum levels in formulations for parenteral administration for patients on dialysis to minimize the risk of aluminum accumulation as these patients may develop osteomalacia. Potential adverse effects of EDTA may also include nephrotoxicity and renal tubular necrosis. Furthermore, EDTA is a chelating agent that is not an approved excipient in some international markets, such as Japan.

The present invention provides a formulation that overcomes these and other problems associated with pharmaceutical formulations. The present invention provides a formulation that requires no antioxidant, contains no additives that would lead to an increase in the levels of aluminum in the formulation, and may be terminally sterilized. It has also been surprisingly discovered that the novel formulations of the invention provide a synergistic preservative effect that could not be predicted from the anti-microbial effect of the alcohol and gylcol derivative as individual agents.

SUMMARY OF THE INVENTION

The present invention provides a pharmaceutical composition comprising a therapeutically effective amount of a therapeutic agent and an organic solvent selected from low molecular weight alcohols and glycol derivatives. The formulations of the invention provide a synergistic preservative effect. The term "synergistic preservative effect" means a preservative effect that is not additive, as would be predicted from the individual effect of each agent, but is instead gives a level of preservation which is above that which would be predicted, i.e., is synergistic. The preservative effect is measured following the guidelines of USP 23.

Preferred embodiments provide compositions comprising vitamin D compounds, ethanol and propylene glycol (PG). More preferred are compositions comprising paracalcitol, ethanol, propylene glycol and further comprising water. Most preferred are compositions comprising paracalcitol, 20% (v/v) ethanol, 30% (v/v) PG, and 50% (v/v) water.

A further embodiment of the invention provides a solution that is suitable to provide a synergistic preservative effect to therapeutic agents dissolved therein.

A further embodiment of the invention provides terminally sterilized formulations of the present invention.

Yet another embodiment of the present invention is a formulation which provides a final dosage form of paracalcitol which contains 5 μg/ml paracalcin, ethanol, propylene glycol, and water.

Processes for preparing such sterile, cosolvent solutions are also disclosed.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. **1** shows the preservative effectiveness of a 20% (v/v) ethanol solution.

FIG. **2** shows the preservative effectiveness of a 30% (v/v) propylene glycol solution.

FIG. **3** shows the predicted preservative effectiveness of a 20% (v/v) ethanol/30% (v/v) propylene glycol solution.

FIG. **4** shows the actual preservative effectiveness of a 20% (v/v) ethanol/30% (v/v) propylene glycol solution.

FIG. **5** shows the preservative effectiveness of a 30% (v/v) ethanol/20% (v/v) propylene glycol solution.

FIG. **6** shows the preservative effectiveness of a 40% (v/v) ethanol/10% (v/v) propylene glycol solution.

DETAILED DESCRIPTION OF THE INVENTION

The present invention provides a self-preserved, stable, formulation of a therapeutic agent in a cosolvent formulation.

The therapeutic agent which can be utilized with the formulations of the present invention may be selected from the entire range of biologically and/or pharmacologically active substances which lack adequate solubility in aqueous systems, that is, agents which lack adequate solubility in water to yield an effective therapeutic dose. The precise biological activity of the substance is immaterial so long as the substance can be dissolved in the present formulations. More preferred are agents which are soluble at less than 1 μg/ml in water. Preferred agents of this subclass are vitamin D compounds, for example, calcitriol and paracalcitol.

The preferred route of administration of the formulations of the present invention is parenteral, most preferred is intravenous.

The term "vitamin D compound" means vitamin D and its derivatives. Exemplary vitamin D compounds are 19-nor-1α,3β,25-trihydroxy-9,10-secoergosta-5,7(E),22(E)-triene (generic name paracalcitol) and 1α,25-dihydroxycholecalciferol (generic name calcitriol).

The term "low molecular weight alcohol" means an aliphatic alcohol of from 1 to 5 carbons, e.g., ethanol, propanol, butanol, etc. Ethanol is listed on the United States Food and Drug Administration's (FDA) list of compounds, which are generally recognized as safe (GRAS), and is therefore preferred in formulations of the present invention intended for administration to humans.

The term "glycol derivative" refers to liquid or solid compounds e.g., glycerin, as well as polymers of glycol, e.g., polyethylene glycol (PEG) and propylene glycol (PG). Preferred for parenteral administration are liquid polymers, typically having molecular weight less than 1,000. The most preferred glycol derivative is PG.

Unless specified to the contrary, the percent concentrations stated herein are on a volume per volume (v/v) basis.

The organic solvent may comprise up to 100% of the excipient in the compositions of the present invention. It will

be appreciated by those skilled in the clinical arts that the amount of organic solvent in the preferred parenteral formulations of the invention should be kept to a minimum. At the same time the requirements of manufacturing and required dosage ranges must be considered to ensure adequate solubility of the vitamin D compound in the present formulations.

Thus, the amount of low molecular weight alcohol may range from zero to 100%, keeping in mind that greater than 50% alcohol may present added expense and difficulties in manufacturing. The preferred range is about 15 to about 50% with the preferred alcohol being ethanol. When the therapeutic agent is paracalcitol, the most preferred are solutions containing 20% ethanol.

The amount of glycol derivative may also vary from zero to 100%. The preferred range is about 15 to about 35%. When the therapeutic agent is paracalcitol, the preferred glycol derivative is propylene glycol at 30%.

When the total amount of organic solvent comprises less than 100% of the volume, the remainder can be made up with water. As it is preferred that the total amount of organic solvent in preparations for parenteral administration be kept to a minimum, the preferred amount of water is 50%.

Thus, the most preferred formulation of the present invention contains about 15 to about 50% ethanol, about 15 to about 50% FPG, and the balance, if needed, water.

The amount of the therapeutic agent in the formulations of the invention is dependent merely on the solubility of the agent in the excipients of the present invention. Those skilled in the art can, without undue experimentation, determine the solubility of any therapeutic agent in the compositions described herein.

The amount of the therapeutic agent is not critical to the present invention and may be varied so as to obtain an amount of the agent that is effective to achieve the desired therapeutic response for a particular patient, composition, and mode of administration. The selected dosage level will depend on the activity of the therapeutic agent, the route of administration, the severity of the condition being treated and the condition and prior medical history of the patient being treated. The preferred therapeutic dose for the preferred vitamin D compounds is between about 2 and about 10 μg/ml, 5 μg/ml being most preferred.

The cosolvent formulations of the present invention provide certain advantages over prior art formulations.

With respect to lipid based formulations of the prior art, the formulations of the present invention are more readily manufactured, both in ease of manufacture and in the omission of steps to prevent peroxide formation in the resulting formulation, and may be more readily tolerated by patients for whom excess lipid exposure is a concern.

In addition, the formulations of the present invention omit the use of surfactants, which can cause irritation at the site of injection in some patients. The formulations of the present invention also avoid the use of buffering agents in order to control the pH of the solution. This is an additional advantage in manufacturing and provides a further advantage of avoiding the source of aluminum in the finished formulation. In addition, the present formulations do not suffer from discoloration due to the use of antioxidants, as such excipients are not required. Thus while buffers, surfactants and additional excipients may be added to the present formulation, such additional components are not critical to achieve the self-preserving feature or to maintain the stability of the therapeutic agents therein.

A further advantage of the formulations of the present invention is that the formulations may be terminally sterilized. With respect to formulations, terminal sterilization generally includes, but is not intended to be limited to, autoclaving, gamma radiation and electron beam sterilization techniques. For purposes of this disclosure, terminal sterilization will primarily refer to autoclaving processes. Of course, aseptic fill techniques may also be employed, however, terminal sterilization is preferred.

Terminal sterilization provides greater sterility assurance level (SAL) (10^{-6}), than that of aseptic filling (10^{-3}). Thus, terminal sterilization of the formulations of the present invention, sterilized by autoclaving, imparts a 10^3 fold increase in SAL of the final product over aseptic filling techniques. Parenteral products manufactured having a high SAL reduce patient exposure to potential infections.

It has been surprisingly discovered that the formulations of the present invention provide yet another advantage over prior art formulations. Ethanol is well known for being a bactericidal and fungicidal agent, although ordinary use would involve ethanol at a concentration in excess of 70%. Ethanol is not used as a preservative per the definition of USP 23 and is not listed as preservative in any drug formulation listed in Physicians Desk Reference (1995). Propylene glycol has been defined as a true preservative and at least one formulation has used this solvent at a concentration of 3%. We have discovered that combinations of these preferred solvents provide an antimicrobial effect greater than that which would be predicted from the additive effect of the solvents. In particular it was discovered that the synergistic effect is observed with respect to at least three of the organisms that are used in the well-recognized test USP 23.

Thus, yet a further advantage provided by the formulations of the present invention is in the ability to package therapeutic agents in packaging, e.g., vials, which are suitable for multiple use.

In the most preferred embodiments of the invention, a paracalcitol formulation for parenteral administration may be supplied in a sterile unit dose flint glass vial or ampoule of 1, 2, or 5 ml. The dosage forms are stable for extended periods and can be stored at temperatures of from about 15° to 30° C.

Each 1 ml of solution preferably contains 5 μg of paracalcitol, 0.2 ml ethanol, 0.3 ml PG, and water for injection q.s.

It is understood by those skilled in the art that all components of the present formulations are of a pharmaceutically acceptable grade and quality.

Ampoules or vials containing the formulations of the present invention may be aseptically filled using a series of filters to assure a sterility assurance level (SAL) of 1×10^{-3}. More preferably, ampoules or vials containing the formulations of the present invention may be filled and then terminally sterilized to provide a SAL of 1×10^{-6}. For example, a solution of a formulation of the present invention may be filtered, using a 0.45 micrometer (μm) or finer membrane filter (Millipore Corporation, Bedford, Mass. 01730), into ampoules. The containers may be sealed and terminally sterilized.

Terminal sterilization of the final product may be done under conditions that are suitable to maintain the stability of the product. Preferably, the formulations are terminally sterilized at an F_0 of about 8 to about 18. The term "F^0" means the integrated lethality or equivalent minutes at 121.11° C. and is well known to those skilled in the art. For example, a F_0 of 8 denotes a sterilization cycle run at 121.11° C., with saturated steam for 8 minutes, while a F_0

of 18 denotes a cycle at 121.110° C., with saturated steam for 18 minutes.

EXAMPLE 1

Solubility of Paracalcitol in a Cosolvent System

An adequate amount of paracalcitol was weighed and added to 10 ml of cosolvent contained in a 10 ml stoppered glass test tube. Two samples were prepared for each cosolvent composition. The test tubes containing samples were shaken in a 25° C. reciprocal shaking water bath at 100 rpm. Upon complete dissolution, an aliquot was filtered through a 0.45 micron syringe filter and the filtrate diluted 1:1 with 50% methanol. The resulting diluted material was measured for the content of paracalcin. Table 1 shows the results of concentration of paracalcin in the listed cosolvent systems.

TABLE 1

Ethanol	Propylene glycol	Water	Percalcitol (μg/ml)
0.00	0.506	0.494	14.21
0.00	0.302	0.698	0.58
0.100	0.257	0.643	2.88
0.199	0.207	0.594	15.94
0.201	0.308	0.491	72.90
0.299	0.00	0.701	6.82
0.347	0.102	0.551	119.37
0.498	0.00	0.502	601.63

EXAMPLE 2

Stability of Paracalcitol in a Cosolvent Formulation

Samples of paracalcitol (5 μg/ml) in 20% ethanol/30% propylene glycol/50% water were prepared for stability testing. In an appropriate vessel, water for injection to approximately 30% of final volume is added. Propylene glycol is added to the vessel with mixing. In a separate container, the specified amount of paracalcitol is dissolved in a portion of the ethanol (190 proof non-beverage), which is obtained from the total volume of ethanol specified for the batch, and added to the vessel with mixing. An additional aliquot of the batch ethanol is used to rinse the container and the rinsing solution is added to the vessel with mixing. The remaining alcohol is added to the vessel with mixing. Q.s. with water for injection to final volume and mix for approximately 30 minutes. The solution is filtered through a 0.45 micron membrane and dispensed into ampuls. Each ampul is flame sealed and autoclaved to F_0 16.

One set of ampuls are tested (T=0) for percent paracalcitol remaining in solution and served as control (i.e., 100% remaining). A second set of ampuls are stored at 40° C. and tested at 1, 2, and 3 months. A final set are stored at 30° C. and tested at 1, 2, 3, 6, 9, 12, 18, and 24 months. The results are shown in Table 2 as paracalcin remaining as a percent of control (T=0). Each time point represents 1 to 5 data points.

TABLE 2

Time (months)	30° C.	40° C.
0 (Initial)	100	100
1	96	96
2	96	96
3	97	98
6	96	n.t.
9	97	n.t.
12	100	n.t.
18	97	n.t.
24	96	n.t.

EXAMPLE 3

Self-preserved Cosolvent Formulations

Solutions of 20% ethanol, 30% propylene glycol, 20% ethanol/30% PG, 30% ethanol/20% PG, and 40% ethanol/10% PG were passed through a 0.45 micron filter and tested by the USP 23 preservative effectiveness test as described in United States Pharmacopoeia 23-NF 18, 1995 Ed., Chapter 51, page 1681, which is incorporated herein by reference. Briefly, this involves inoculating the test solution with 10^5 to 10^6 test organisms per milliliter and then determining the number of surviving organisms after 7, 14, 21, and 28 days incubation at 20–25° C. using standard microbiological methods. Day 0 data is not required by USP 23 but was included in this study. A filtration and wash method was used to remove the inactivating agents for purposes of recovering the microorganisms, but other equivalent methods can also be validated for use. The USP test organisms include the bacteria *Staphylococcus aureus, Escherichia coli,* and *Pseudomonas aeruginosa,* a yeast (*Candida albicans*), and a mold (*Aspergillus niger*). In order to meet the criteria of the USP 23 preservative effectiveness test, the bacteria must demonstrate a 90% (1 logarithmic) reduction at Day 7 and a 99.9% (3 logarithmic) reduction at Day 14 from the initial inoculum level. The yeast and mold must not increase from the initial inoculum level. The initial inoculum level can either be calculated by knowing the stock culture concentration or by using a buffer control instead of the test solution.

As can be seen by reference to FIGS. **1** and **2**, both the solution of 20% ethanol and 30% propylene glycol meet the acceptance criteria of the USP 23 preservative effectiveness test. With respect to the mold *Aspergillus niger,* it is noted that neither solvent provides complete elimination of the microorganism; 20% ethanol is inhibitory and 30% PG has very little effect. As stated above, this result are not entirely unexpected as both of these solvents are recognized in the art as antimicrobial agents.

As both propylene glycol and alcohol share antimicrobial and solvent properties, the preservative effect of this cosolvent system would be predicted to be sum of the individual efficacies of ethanol and propylene glycol (H. Takruri and C. B. Anger, Preservation of Dispersed Systems, pp. 85, 101 in Pharmaceutical Dosage Forms; Dispersed Systems, H. A. Lieberman, M. M. Reiger, and G. S. Banker, Ed. (1989)). FIG. **3** shows this predicted effect of a solution of 20% ethanol in combination with 30% propylene glycol, as determined by the sum of values generated for FIGS. **1** and **2**. However, FIG. **4** shows the unexpected result of the actual preserving effect that a combination of 20% ethanol and 30% propylene glycol is not additive, but is synergistic. The mold, *A. niger,* is completely killed, i.e., the number of microorganisms remaining is below the detection limits of the assay, by the cosolvent within the 7 days. The addition of paracalcin to the 20% ethanol/30% PG formulation has no effect on the preservative effect of the formulation (data not shown).

FIGS. **5** and **6** demonstrate that the ratio of ethanol and PG is not critical to the self-preserving properties of this cosolvent formulation.

We claim:

1. A sterilized, self-preserved, aqueous pharmaceutical composition for parenteral administration consisting essentially of a therapeutically effective amount of a vitamin D compound, about 50% (v/v) of an organic solvent selected from the group consisting of low molecular weight alcohols in the range of about 15% to about 30% (v/v) and glycol derivatives in the range of about 20% to about 35% (v/v), and about 50% (v/v) water.

2. The composition of claim **1** wherein the low molecular weight alcohol is ethanol.

3. The composition of claim **1** wherein the glycol derivative is selected from the group consisting of glycerin and propylene glycol.

4. The composition of claim **3** wherein the glycol derivative is propylene glycol.

5. The composition of claim **1** wherein the vitamin D compound is selected from the group consisting of parecelcitol and calcitriol.

6. The composition of claim **1** wherein the low molecular weight alcohol is ethanol and the glycol derivative is propylene glycol.

7. The composition of claim **6** wherein the vitamin D compound is paracalcitol or calcitriol.

8. The composition of claim **7** wherein the vitamin D compound is present between about 2 μg/ml and about 10 μg/ml.

9. The composition of claim **8** wherein the vitamin D compound is present at about 5 μg/ml.

10. The formulation of claim **1** wherein sterilization is terminal sterilization.

11. The formulation of claim **1** wherein the sterilization is aseptic fill.

12. A sterilized, self-preserved pharmaceutical formulation for parenteral administration consisting essentially of:

2–10 micrograms/milliliter of a paracalcitol;

20% (v/v) ethanol;

30% (v/v) propylene glycol; and

50% (v/v) water.

13. A process for preparing a pharmaceutical formulation comprising a therapeutically effective amount of paracalcitol comprising the steps of:

a) preparing a mixture of water and propylene glycol;

b) preparing a mixture of paracalcitol and ethanol;

c) combining the mixtures from steps (a) and (b) to prepare a uniform mixture; and

d) filtering said uniform mixture.

14. A unit dose of a sterile, self-preserved pharmaceutical formulation for parenteral administration consisting essentially of:

2–10 micrograms/milliliter of paracalcitol;

20% (v/v) ethanol;

30% (v/v) propylene glyco; and

50% (v/v) water.

* * * * *

UNITED STATES PATENT AND TRADEMARK OFFICE

CERTIFICATE OF CORRECTION

PATENT NO. : 6,136,799
DATED : October 24, 2000
INVENTOR(S) : Lukchiu Li et al.

It is certified that error appears in the above-identified patent and that said Letters Patent is hereby corrected as shown below:

Column 2,
Line 5, replace "paracalcin" with -- paracalcitol --.

Column 3,
Line 26, replace "FPG" with -- PG --.

Column 4,
Line 63, replace "F^{0}" with -- F_0 --.

Column 5,
Line 1, replace "121.110º" with -- 121.11º --.
Line 14, replace "paracalcin." with -- paracalcitol. --.
Line 15, replace "paracalcin" with -- paracalcitol --.

Column 6,
Line 63, replace "paracalcin" with -- paracalcitol --.

Column 7,
Line 8, replace "parecelcitol" with -- paracalcitol --.

Signed and Sealed this

Ninth Day of September, 2003

JAMES E. ROGAN
Director of the United States Patent and Trademark Office